Original investigation

Aldosterone Synthase Gene (CYP11B2) Polymorphism in Korean End-Stage Renal Disease Patients on Hemodialysis

Ji Eun Lee, M.D.[1], So Yon Bae, B.A.[2], Jeong-Yup Kim, M.D.[3], Heui Jung Pyo, M.D.[3], Western Dialysis Physician Association (WDPA) and Young Joo Kwon, M.D.[3]

[1]Department of Internal Medicine, Wonkwang University College of Medicine, Iksan, Korea
[2]Institute of Kidney Disease Research, [3]Department of Internal Medicine,
Korea University College of Medicine, Seoul, Korea

Aldosterone synthase gene (CYP11B2) −344C/T polymorphism has been reported to be associated with serum aldosterone level, urinary aldosterone excretion, blood pressure, and left ventricular size and mass. The aim of this study was to evaluate the relation between CYP11B2 polymorphism and end-stage renal disease (ESRD) in the Korean population and the association with CYP11B2 polymorphism and cardiovascular morbidity in ESRD patients on hemodialysis. Genotyping was performed in 134 control subjects and 271 ESRD patients for CYP11B2 polymorphism using polymerase chain reaction through subsequent cleavage with restriction enzyme. Also current blood pressure, demographic, anthropometric and biochemical variables were investigated. The genotype distribution did not differ between ESRD patients and controls and there were no significant differences in blood pressure, use of antihypertensive medication, left ventricular hypertrophy and cardiovascular disease among the three genotypes in ESRD patients on hemodialysis. Our findings do not support the hypothesis that CYP11B2 polymorphism may be associated with prevalence of ESRD and suggest that CYP11B2 polymorphism may not be a genetic marker for cardiovascular morbidity in Korean ESRD patients.

Electrolyte Blood Press 7:67-72, 2009 • doi: 10.5049/EBP.2009.7.2.67

Key Words: aldosterone synthase; polymorphism, genetic; renal dialysis

Introduction

Renal function and blood pressure are tightly linked and hypertension *per se* is a risk factor for the development of end-stage renal disease (ESRD)[1, 2]. The renin-angioten-sin-aldosterone system (RAAS) is a key regulator of both blood pressure and renal function, so that genes encoding components of the RAAS can be candidate genes for evaluating predisposition for the development of hypertension, cardiovascular disease and progression of renal disease. Polymorphisms have been described in the genes encoding several important components of the RAAS, including angiotensinogen[3], angiotensin-converting enzyme[4], angiotensin type Ⅰ receptors[5], and aldosterone synthase[6].

The aldosterone synthase gene, CYP11B2, encodes for a cytochrome P450 enzyme, involved in the terminal steps of aldosterone synthesis in the zona glomerulosa cells of human adrenal glands and its expression is regulated by angiotensin II and potassium[7]. The CYP11B2 −344C/T polymorphism, which is located at a putative binding site for the steroidogenic transcription factor (SF-1), has been

Received October 26, 2009. Revised November 23, 2009. Accepted November 23, 2009
Corresponding author: Young Joo Kwon, M.D.
Korea University Guro Hospital, 97 Gurodong-Gil, Guro-Gu, Seoul, 152-703, Korea
Tel: +82-2-2626-3036, Fax: +82-2-2626-1077
E-mail: yjkwon@korea.ac.kr

reported to be associated with serum aldosterone level[8], urinary aldosterone excretion[9], blood pressure[9-12], left ventricular size and mass[13, 14]. However, there were few studies on the association of CYP11B2 −344C/T polymorphism and renal function, which has inconsistent results[15, 16]. The aim of this study is to evaluate the relation between CYP11B2 polymorphism and ESRD in the Korean population and the association with CYP11B2 polymorphism and cardiovascular morbidity in ESRD patients on hemodialysis.

Methods

1. Subjects

The study subjects were 271 ESRD patients on maintenance hemodialysis over three months from dialysis centers located in the western district of Seoul, Korea and 134 control subjects without renal disease from Korea University Guro Hospital. The controls were individuals who have had no medical history and were normal in blood pressure, blood chemistry, urinalysis, and electrocardiogram (EKG).

Basic demographic data, current blood pressure, information on underlying renal disease, previous cardiovascular disease, and current antihypertensive medication were obtained for all ESRD subjects. Left ventricular hypertrophy was determined as the voltage sum SV1+ (RV5 or RV6) $\geq$35 mm using the Sokolow-Lyon voltage criteria on EKG[17]. Clinical cardiovascular diseases include ischemic heart disease, cerebrovascular disease and congestive heart failure. Ischemic heart disease was considered if the patient had previous myocardial infarction, positive coronary angioplasty or other diagnostic procedure (e.g. exercise test, thallium or dobutamine stress test) or the presence of ischemic change on the resting EKG (as distinct from left ventricular hypertrophy). Cerebrovascular disease was established if the patient had a history of transient ischemic attacks or stroke verified by computed tomography, or carotid artery stenosis greater than 70% verified by doppler ultrasound. Congestive heart failure was defined as clinical evidence of pulmonary edema, not attributable to errors in fluid balance, and/or moderate to severe left ventricular dysfunction on echocardiography (left ventricular ejection fraction <45%). In addition, biochemical data including plasma hemoglobin, hematocrit, serum albumin, creatinine, glucose, total cholesterol, total calcium, phosphorus, intact parathyroid hormone, and single-pool Kt/V were obtained.

2. Genotyping

Genomic DNA was extracted from peripheral blood using the DNA extraction kit (G-Dex$^{TM\ IIb}$ No.17241, iNtRON). Genotypes were determined by polymerase chain reaction (PCR) amplification of the promoter region of the CYP11B2 gene using the oligonucleotide primers (upstream: 5'-CAG GAGGAGACCCCATGTGAC-3'; downstream: 5'-CCTCC ACCCTGTTCAGCCC-3'). PCR conditions were: initial denaturation at 94˚C for 3 min; then 32 cycles at 94˚C for 1 min, at 60˚C (annealing) for 1 min, and at 72˚C (extension) for 1 min. Restriction fragment length polymorphism (RFLP) was performed by adding 10 U of restriction endonuclease *Hae*III site in the appropriated buffer to 5 μL from each reaction (a 537 bp product) and by incubating at 37˚C for 2 hours. The samples digested then underwent electrophoresis on 2.5% agarose gel with a Gel Electrophoresis Apparatus, ethidium bromide stained, and analyzed under UV lights. Since the (−344)T allele lacks an *Hae*III site (GGCC) present in the (−344)C allele, the (−344)T alleles are detected as fragments of 273 bp and (−344)C alleles as fragments of 202 bp (plus smaller fragment in each case) (Fig. 1).

3. Statistical analysis

Statistical analysis was performed using the Statistical Package for Social Sciences (SPSS-12.0, Chicago, Illinois,

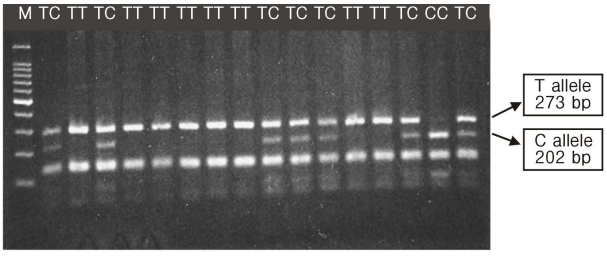

Fig. 1. CYP11B2 polymorphism. CYP11B2, aldosterone synthase.

USA). Values are expressed as mean±standard deviation (SD) or percentage. Statistical differences between means were assessed by t test or analysis of variance (ANOVA) for analysis of continuous variables. For categorical variables, the 2×2 contingency table χ^2 test was used. To examine the independent contribution of CYP11B2 polymorphism and estimate the odds ratio, while adjusting for the effects of other clinical characteristics, we used a logistic analysis. The Hardy-Weinberg equilibrium was tested by a χ^2 test. *P* values below 0.05 were considered statistically significant.

Results

The baseline characteristics of controls and ESRD patients are summarized in Table 1. The mean age and male prevalence were similar between controls and ESRD patients. Genotype and allele frequencies for the polymorphism of CYP11B2 in controls and ESRD patients are presented in Table 2. The controls and ESRD patients were in Hardy-Weinberg equilibrium for the polymorphism. Genotype distribution of CYP11B2 polymorphism did not differ between controls and ESRD patients.

Table 3 shows the comparison of clinical and biochemical characteristics of ESRD patients according to genotypes of CYP11B2 polymorphism. We could observe the differences in systolic blood pressure and frequency of diabetes among the three genotypes in this analysis. However, these findings were not significant after adjustment for age and sex. In addition, there was no association of CYP11B2 polymorphism with left ventricular hypertrophy or cardiovascular disease in ESRD patients (Table 4).

Table 1. Baseline Characteristics of Controls and End-Stage Renal Disease Patients

Characteristics	Controls (n=134)	Patients (n=271)
Age (years)	53.0±10.7	54.1±13.2
Sex (male%/female%)	55.0/45.0	54.2/45.8
Body mass index (kg/m^2)	23.1±2.9	21.5±3.1
Hemodialysis duration (years)	-	4.3±3.6
Cause of renal failure (%)	-	
Diabetic nephropathy		36.9
Hypertensive nephrosclerosis		20.3
Chronic glomerulonephritis		8.5
Polycystic kidney disease		3.0
Others		1.8
Unknown		22.5
Smoking (%)	NA	20.8
Systolic blood pressure (mmHg)	116.0±12.3	156.1±21.2
Diastolic blood pressure (mmHg)	70.8±8.8	90.3±7.3
Use of antihypertensive drugs (%)	0	73.1
0 / 1 / 2 /		26.9/17.0/22.9/
3 / 4 / 5		18.8/13.7/0.7
Left ventricular hypertrophy (%)	0	35.3
Cardiovascular disease (%)	0	20.7
Hemoglobin (g/dL)	13.6±1.5	10.2±1.2
Hematocrit (%)	41.9±3.9	31.2±3.7
Serum albumin (g/dL)	4.9±0.2	4.0±0.3
Serum creatinine (mg/dL)	0.9±0.2	9.9±2.7
Serum glucose (mg/dL)	93.3±10.4	121.6±54.9
Serum total cholesterol (mg/dL)	187.4±32.2	149.0±33.0
Serum total calcium (mg/dL)	9.6±0.4	8.8±0.8
Serum phopshorus (mg/dL)	3.7±0.4	5.3±1.7
Intact parathyroid hormone (pg/mL)	NA	118.6±159.5
Kt/V	-	1.5±0.3

NA, Not available.

Discussion

Our study was designed to test the hypothesis that the prevalence of renal failure may be influenced by gene polymorphism of the RAAS, especially aldosterone synthase gene polymorphism. In this cross-sectional study, we could not observe significant differences in the genotype and allele frequency of the CYP11B2 −344C/T polymorphism between controls and ESRD patients. Previous studies performed in Europe showed inconsistent results. Lovati et

Table 2. Genotype and Allele Frequencies of the CYP11B2 Polymorphism in Controls and ESRD Patients

	Controls (n=134)	ESRD (n=271)	
	N (%)	N (%)	OR* (95% CI)
Genotype			
TT	59 (44.0)	130 (48.0)	1.00 (reference)
TC	64 (47.8)	115 (42.4)	0.82 (0.53-1.26)
CC	11 (8.2)	26 (9.6)	1.04 (0.48-2.26)
			P=0.613
Allele			
%T	0.68	0.69	1.00 (reference)
%C	0.32	0.31	0.94 (0.68-1.28)
			P=0.675

CYP11B2, aldosterone synthase; ESRD, end-stage renal disease.
*Adjusted odds ratio for age and sex.

Table 3. Comparison of Clinical and Biochemical Characteristics of End-Stage Renal Disease Patients according to CYP11B2 Polymorphism

	TT (n=130)	TC (n=115)	CC (n=26)	*P*
Age (years)	53.9±13.7	53.6±13.1	57.8±11.7	0.326
Sex (%male)	51.5	55.7	61.5	0.597
Body mass index (kg/m^2)	21.4±3.0	21.6±3.4	21.4±3.0	0.918
Hemodialysis duration (years)	4.5±3.4	4.3±3.9	3.4±2.8	0.359
Diabetes (%)	26.2	40.0	42.3	0.044
Smoking (%)	19.8	23.1	16.0	0.689
Systolic blood pressure (mmHg)	158.4±19.7	152.2±23.0	162.2±17.3	0.041
Diastolic blood pressure (mmHg)	90.9±7.3	89.7±7.7	90.4±5.6	0.515
Use of antihypertensive drugs (%)	74.6	70.4	76.9	0.684
Left ventricular hypertrophy (%)	30.2	37.8	48.0	0.181
Previous cardiovascular disease (%)	19.2	20.9	26.9	0.675
Hemoglobin (g/dL)	10.3±1.1	10.1±1.2	9.7±1.1	0.057
Serum albumin (g/dL)	4.1±0.3	4.0±0.3	3.9±0.2	0.067
Serum creatinine (mg/dL)	9.9±2.9	9.8±2.7	10.4±2.1	0.688
Serum total cholesterol (mg/dL)	152.5±32.6	146.3±32.8	149.8±35.7	0.354
Serum total calcium (mg/dL)	8.8±0.9	8.8±0.7	8.8±0.8	0.936
Serum phosphorus (mg/dL)	5.4±1.9	5.2±1.7	5.1±1.3	0.659
Intact parathyroid hormone (pg/mL)	123.0±182.5	116.7±124.5	105.8±174.9	0.882
Kt/V	1.5±0.3	1.4±0.3	1.4±0.2	0.156

CYP11B2, aldosterone synthase.

Table 4. Adjusted Odds Ratio for Left Ventricular Hypertrophy and Cardiovascular Disease by CYP11B2 Genotype

	Left ventricular hypertrophy	Cardiovascular disease
Genotype	OR* (95% CI)	OR* (95% CI)
TT	1.00 (reference)	1.00 (reference)
TC	1.41 (0.75-2.66)	1.00 (0.50-2.01)
CC	1.78 (0.67-4.70)	1.16 (0.40-3.32)
	P=0.393	*P*=0.960

CYP11B2, aldosterone synthase.
*Adjusted odds ratio for age, sex, body mass index, hemodialysis duration, smoking, hypertension, diabetes, hemoglobin, serum albumin and total cholesterol levels.

al. reported that there was no association between the CYP11B2 genotype and progression of renal failure among the ESRD patients[15]. On the other hand, Fabris et al. report ed that significant association was found between the CYP11B2 gene polymorphism and renal insufficiency in the hypertensive population[16]. They observed an increased proportion of CC genotype in hypertensive patients with renal damage compared with hypertensive patients without renal damage. The adjusted odds ratio was 3.89 for CYP11B2 −344C allele as a recessive effect. However, CYP11B2 genotypes were not in Hardy-Weinberg equilibrium among controls in Fabris and colleagues' report, so linkage disequlibrium in control subjects weakens a causal interpretation of these statistically significant findings. Controls in our study are of the normotensive healthy population and show similar allele frequency in comparison with another Korean study (T allele frequency 0.69)[18]. In the Korean population, there has been research aimed at evaluating the association between CYP11B2 polymorphism and hypertension[18], myocardial infarction[19], and risk of coronary in-stent restenosis[20]. To our knowledge, this is the first report about the CYP11B2 C−344T allele frequency in Korean ESRD patients.

Several studies of the association between this polymorphism and hypertension[12, 21, 22], left ventricle size and mass[13, 23], arterial stiffness[24], and myocardial infarction[14, 25] in the general population and hypertensive individuals with normal renal function have been performed. In ESRD patients, however, studies of association of CYP11B2 −344C/T polymorphism and left ventricular hypertrophy and cardiovascular morbidity are few. Our results did not show significant differences in left ventricular hypertrophy by EKG criteria and cardiovascular disease among the three genotypes in ESRD patients. We think this lack of association is due to not only limitation of the study, but

also multifactorial etiology of cardiovascular morbidity in ESRD patients. The limitations of this present study are the insufficient statistical power as a result of a relatively small number of patients and the use of the EKG instead of echocardiographic examination for diagnosis of left ventricular hypertrophy. We obtained age and sex adjusted odds ratio using logistic regression methods because we could not match each individual case to his or her own control. It is also possible that the ESRD patients with high risk genotype may be excluded from the present study because of premature mortality due to cardiovascular influences by CYP11B2 polymorphism. Thus, further prospective investigation with sufficient statistical power is needed to explore the role of CYP11B2 polymorphism in the susceptibility of ESRD and cardiovascular effect in ESRD patients.

In conclusion, our findings do not support the hypothesis that CYP11B2 polymorphism is associated with prevalence of ESRD and suggest that CYP11B2 polymorphism may not be a genetic marker for cardiovascular morbidity in Korean ESRD patients.

Western Dialysis Physician Association (WDPA)

Keong Wook Kim, M.D., Sang Wook Kim, M.D., Seong Nam Kim, M.D., Seung-Jung Kim, M.D., Yung A Kim, M.D., Hong Ryul Kim, M.D., Hwa Jung Kim, M.D., Jung Woo Noh, M.D., Seung Hwan Son, M.D., Keong Sik Oh, M.D., Kyun Il Yoon, M.D., Young Ki Lee, M.D., Yung Chun Lee, M.D., Jong Young Lee, M.D., Hun Kwan Lim, M.D., No Won Jeon, M.D., Seong Tae Jo, M.D., Byoung Chunn Jeoung, M.D. and, Kyu Bok Choi, M.D.

Acknowledgements

This study was supported by the Extramural grant RO605641.

References

1) Perry HM, Jr., Miller JP, Fornoff JR, et al.: Early predictors of 15-year end-stage renal disease in hypertensive patients. Hypertension 25:587-594, 1995
2) Klag MJ, Whelton PK, Randall BL, et al.: Blood pressure and end-stage renal disease in men. N Engl J Med 334:13-18, 1996
3) Hata A: Role of angiotensinogen in the genetics of essential hypertension. Life Sci 57:2385-2395, 1995
4) Niu T, Chen X, Xu X: Angiotensin converting enzyme gene insertion/deletion polymorphism and cardiovascular disease. Drugs 62:977-993, 2002
5) Schmidt S, Beige J, Walla-Friedel M, Michel MC, Sharma AM, Ritz E: A polymorphism in the gene for the angiotensin II type 1 receptor is not associated with hyperension. J Hypertens 15:1385-1388, 1997
6) Davies E, Kenyon CJ: CYP11B2 polymorphisms and cardiovascular risk factor. J Hypertens 21:1249-1253, 2003
7) Clyne CD, Zhang Y, Slutsker L, Mathis JM, White PC, Rainey WE: Angiotensin II and potassium regulate human CYP11B2 transcription through common cis-elements. Mol Endocrinol 11:638-649, 1997
8) Russo P, Siani A, Venezia A, et al.: Interaction between the C(-344)T polymorphism of CYP11B2 and age in the regulation of blood pressure and plasma aldosterone levels: cross-sectional and longitudinal findings of the Olivetti Prospective Heart Study. J Hypertens 20:1785-1792, 2002
9) Davies E, Holloway CD, Ingram MC, et al.: Aldosterone excretion rate and blood pressure in essential hypertension are related to polymorphic differences in the aldosterone synthase gene CYP11B2. Hypertension 33:703-707, 1999
10) Kumar NN, Benjafield AV, Lin RC, Wang WY, Stowasser M, Morris BJ: Haplotype analysis of aldosterone synthase gene (CYP11B2) polymorphisms shows association with essential hypertension. J Hypertens 21:1331-1337, 2003
11) Brand E, Chatelain N, Mulatero P, et al.: Structural analysis and evaluation of the aldosterone synthase gene in hypertension. Hypertension 32:198-204, 1998
12) Tamaki S, Iwai N, Tsujita Y, Kinoshita M: Genetic polymorphism of CYP11B2 gene and hypertension in Japanese. Hypertension 33:266-270, 1999
13) Kupari M, Hautanen A, Lankinen L, et al.: Associations between human aldosterone synthase (CYP11B2) gene polymorphisms and left ventricular size, mass, and function. Circulation 97:569-575, 1998
14) White PC, Hautanen A, Kupari M: Aldosterone synthase (CYP11B2) polymorphisms and cardiovascular function. Endocr Res 24:797-804, 1998
15) Lovati E, Richard A, Frey BM, Frey FJ, Ferrari P: Genetic polymorphisms of the renin-angiotensin-aldosterone system in end-stage renal disease. Kidney Int 60:46-54, 2001
16) Fabris B, Bortoletto M, Candido R, et al.: Genetic polymorphisms of the renin-angiotensin-aldosterone system and renal insufficiency in essential hypertension. J Hypertens 23:309-316, 2005
17) Sokolow M, Lyon TP: The ventricular complex in left ventricular hypertrophy as obtained by unipolar precordial and limb leads. Am Heart J 37:161-186, 1949
18) Kang BY, Bae JS, Kim KT, Lee KO: DNA polymorphisms of the human CYP11B2 and γ subunit of ENaC genes in Korean hypertensives. Environ Mutagen Carcinog 22:223-

228, 2002
19) Ryu SK, Park HY, Im EK, et al.: The effects of an aldosterone synthase (CYP11B2) gene polymorphism on the risk of myocardial infarction. Korean Circ J 31:1261-1266, 2001
20) Ryu SK, Cho EY, Park HY, et al.: Renin-angiotensin-aldosterone system (RAAS) gene polymorphism as a risk factor of coronary in-stent restenosis. Yonsei Med J 43:461-472, 2002
21) Sookoian S, Gianotti TF, Gonzalez CD, Pirola CJ: Association of the C-344T aldosterone synthase gene variant with essential hypertension: a meta-analysis. J Hypertens 25:5-13, 2007
22) Cheng X, Xu G: Association between aldosterone synthase CYP11B2 polymorphism and essential hypertension in Chinese: a meta-analysis. Kidney Blood Press Res 32:128-140, 2009
23) Sookoian S, Gianotti TF, Pirola CJ: Role of the C-344T aldosterone synthase gene variant in left ventricular mass and left ventricular structure-related phenotypes. Heart 94:903-910, 2008
24) Pojoga L, Gautier S, Blanc H, et al.: Genetic determination of plasma aldosterone levels in essential hypertension. Am J Hypertens 11:856-860, 1998
25) Hautanen A, Toivanen P, Manttari M, et al.: Joint effects of an aldosterone synthase (CYP11B2) gene polymorphism and classic risk factors on risk of myocardial infarction. Circulation 100:2213-2218, 1999