Progress in Nutrition 2019; Vol.21, N. 4: 783-792 DOI: 10.23751/pn.v21i4.7998

Effect of vitamin D receptor fokI gene polymorphism on chronic renal disease

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Summary. Many factors play a role in the progression of Chronic renal disease. Many polymorphisms have been detected in vitamin D receptor gene. FokI polymorphism is one of these. We aimed to present the role played by FokI gene polymorphism in CRD have been examined. Three study groups were formed as group 1 with a total of 60 patients with RRT need with ages ranging between 59,43±12,58, group 2 with a total of 60 patients with GFR decrease of >4 ml/min/year and ages ranging between 59,73±13,14 and group 3 with a total of 60 patients with stable GFR level during follow up period with ages ranging between 63,50±12,21. A total of 77 individuals without CRD and with ages ranging between 41.78±14,28 were included as the control group of the study during polyclinic controls. The frequency of FokI polymorphism FF, Ff and ff genotypes were determined as 51.7%, 35.0% and 13.3% respectively in group 1, as 50.0%, 41.7% and 8.3% respectively in group 2 and as 45.0%, 48.3% and 6.7% respectively in group 3. Whereas the frequency of FF, Ff and ff genotypes were determined respectively as %51.9, %39.0 and %9.1. Prevalence of CRD in all genotypes was determined to be statistically significant for all groups (p>0.05). No relationship was determined between FokI polymorphism and risk factors. In conclusion, it was observed that FokI polymorphism is not related with CRD and risk factors.

Keywords Chronic renal disease, fokI polymorphism, renal replacement therapy, rapid progression

Introduction

Chronic renal disease (CRD) is a syndrome characterized by the progressive loss of kidney functions due to various reasons. Glomerular filtration rate (GFR) generally reduces over the months or years and this reduction varies significantly according to the underlying reason (1). The prevalence of chronic renal disease has increased rapidly during the last decade. It is estimated that the glomerular filtration rate of 13 % of the adult population in the United States of America is lower than 60 ml/min/1.73 m^2. Prevalence in individuals aged 65 and above was determined to vary between 38-44 % (2, 3).

Vitamin D and its active metabolite of 1,25-dihidroksi vitamin D3 (1,25OHD, calcitriol) is one of the well known regulators of cell growth and differentiation. It has been reported in recent studies that vitamin D is related with bone and calcium metabolism control in addition to many biological processes such as immune response formation, metastasis, angiogenesis and apoptosis. In addition, it also known that 1,25O HD stimulates the expression of the oxidative enzymes which are cytochrome P450 family members (4).

Vitamin D shows its effect by interacting with vitamin D receptor (VDR) which is a member of the nuclear receptor gene family. VDR has been coded by a large gene (>100 kb) on chromosome 2q12-14

(5). Allele variations of VDR differing between ethnic groups and races have been defined (6). First studies on VDR polymorphisms were carried out using bone metabolism parameters and especially osteoporosis (7). The relationship between VDR gene polymorphisms and other diseases including cancer and immune system diseases have been put forth in later studies (8). In general, majority of the VDR gene polymorphisms have been determined in regulator regions such as 5' promoter region and 3' UTR region instead of exon coding region.

(T/C) polymorphism is found in the first potential start region. This polymorphism synthesizes a longer VDR protein comprised of 427 amino acids by way of producing an additional start codon. This polymorphism is named as f allele. The existence of f allele is related with less transcriptional activity (5).

The FokI polymorphism on Exon 2 is a functional polymorphism. It is considered as normal when the transcription starts from the first ATG sequence at the restriction allele (f), if there is no restricton (F allele) transcription starts from the next ATG sequence (transcript) and a shorter but functional allele formation takes place. ATG is transformed into ACG as a result of the T C transformation in the start codon of ATG and translation starts from the second ATG. As a result, the 424 aminoacid long VDR protein is synthesized (F allele). Translation starts from the first ATG in cases when there is no T C transformation and the VDR protein that is 3 aminoacids longer (427 aminoacids, f allele) is synthesized (9).

The purpose of this study was to examine the genetic role of VDR FokI polymorphism along with demographic, clinical and laboratory variables on rapid progression with conservative therapy and RRT requirement in chronic renal disease stage 3-5 patients in the Turkish society.

Patients and methods

Formation of the Patient and Control Groups

In this study, patients aged between 18-80 with GFR <60 ml/dk/1.73m^2 who have been included in the chronic renal disease training program after application to the Antalya Education and Research Hospital Nephrology polyclinic have been scanned retrospectively. The patients were classified into groups according CRD staging based on the GFR values. Demographic, clinical and laboratory data on the patients were acquired from the follow-up folders. Three study groups were formed as group 1 with a total of 60 patients with RRT need during follow-up with ages ranging between 59,43±12,58, group 2 with a total of 60 patients with GFR decrease of >4 ml/min/year and ages ranging between 59,73±13,14 and group 3 with a total of 60 patients with stable GFR level during follow up period with ages ranging between 63,50±12,21. Patients followed up for a period of less than 3 months were not included in the study. A total of 77 individuals without CRD and with ages ranging between 41.78±14,28 were included as the control group of the study during polyclinic controls. Approval from the local ethics council was obtained prior to starting the study and written approvals were taken from all individuals in both the control and patient groups after explaining the purpose and procedure of the study (No: 4/6).

DNA isolation

Two milliliter blood samples were drawn from the individuals who participated in the study and placed in tubes containing K_3-EDTA. The blood samples were stored at -80 °C until DNA isolation. Afterwards, the frozen samples were defroested at room temperature and DNA isolation was carried out via QIAquick PCR Purification Kit (250) (Cat no:28106) (Hilden, Germany) with the standard method. The isolated samples were spectrophotometrically measured via NanoDrop and DNA quantitation was made.

Amplification of DNA polymerase chain reaction

The primers used in the study have been designed using the ExonPrimer (http://ihg.gsf.de/ihg/ExonPrimer.html) software. The primers were synthesized by the Sentromer (Istanbul, Turkey) company as 20 pmol.

FOK1-rs222857-F	AGCTGGCCCTGGCACTGACTC
FOK1-rs222857-R	ATGGAAACACCTTGCTTCTTCTCCCTC

The following PCR cycle has been used in the study.

At 95°C	15 Minutes		
At 95°C	45 Seconds		
At 58°C	45 Seconds	⇒	40 repetitions
At 72°C	1 Minute		
At 72°C	10 Minutes		
At 4°C	infinite		

Exonuclease1 and Shrimpalkalen phosphase enzyme mixtures were used to inactivate the excess primers and dNTPs remaining after PCR. Sequencing reaction was carried out using the final products processed via Exo1-SAPIT and BigD Terminator v3.1 and standard protocols.

Sequencing clean up was carried out using Sephadex and Qiagene Sequencing cleanup columns. 3,5 gr Sephadex (Merck G-50 medium) was dissolved in 50 ml water and 600 µl solution was placed in each column. Water was removed via centrifugation at 2500 g for 2 minutes. Products subject to sequencing reaction were placed on the remaining sephadex on the column and the products were cleaned via centrifugation at 2500 g for 5 minutes. The product that is about 30 µl was transferred onto the optical plate and sequencing reaction was carried out by loading to ABI 3130 device. Seq Scape2.5 software was used for analyzing the sequencing reactions.

Statistical evaluation

SPSS 16.0 package software was used for the biostatistical evaluation of the data obtained as a result of the study. Multivariate analysis of variance (ANOVA) was used for evaluating the variables between the control and patient groups and the genotypes for biochemical parameters, Tukey HSD multiple comparisons test was used for comparing the factors. c^2 test was used for the nonparametrik tests, the results were given as average ± standard deviation and $p<0.05$ were accepted as statistically significant.

Results

Characteristic properties for the individuals in CRD groups have been given in Table 1. Similar values were observed in all three groups with regard to age averages and gender difference. Patient diagnosis distribution among the groups was also similar and no statistically significant difference was observed between the groups. Whereas no statistically significant difference was observed between the groups with regard to systolic blood pressure a statistically significant difference was observed between the diastolic pressure values of the 1st and 2nd Groups in comparison with the 3rd Group (respectively, $p<0.05$ and $p<0.001$). The 1st group was observed to be different at a statistically significant level in comparison with the other two groups with regard to basal creatinine and basal GFR ($p<0.001$). No statistically significant difference was determined between groups 2 and 3 with regard to the basal creatinine values, however basal GFR values were observed to be higher in group 2 at a statistically significant level ($p<0.05$). It was observed upon comparing groups 2 and 3 with group 1 that the basal calcium values of group 1 (respectively, $p<0.05$ and $p<0.01$), basal phosphorous, parathormon and hemoglobin values were lower at a statistically significant level ($p<0.001$). On the other hand, no statistically significant difference was observed between groups 2 and 3 as a result of these measurements. Basal proteinuria value was observed to be higher in group 1 at a statistically significant level in comparison with other groups (respectively, $p<0.05$ and $p<0.001$) while the values of group 2 were observed to be higher at statistically significant levels when compared with those of group 3 ($p<0.01$). It was observed when the nonparametric tests were examined that ACE/ARB use was low in Group 1 contrary to the other two groups ($p<0.01$), whereas eritropoetin use was determined to be high at a statistically significant level ($p<0.001$). No statistically significant differences were observed between the groups with regard to the use of vitamin D, atorvastatin and antiacidose. Average follow up times in the groups actualized as 9, 18 and 16 months respectively.

Genotype and allele frequencies for vitamin D receptor and FokI gene of the patient and control groups have been given in Table 2. It was determined that the frequency of FF, Ff and ff genotypes of individuals with chronic renal disease were 51.7 % (n=31), 35.0 % (n=21) and 13.3 % (n=8) respectively for Group 1, 50.0 % (n=30), 41.7 % (n=25) and 8.3 % (n=5) respectively for Group 2 and 45.0 % (n=27), 48.3 % (n=29) and 6.7 % (n=4) respectively for Group 3 (Table 2). Whereas the FF, Ff and ff genotype frequencies for

Table 1 Characteristic properties of the study groups

Patient characteristics	1. Group	2. Group	3. Group
n	60	60	60
Age (years)	59,43 ± 12,58	59,73 ± 13,14	63,50 ± 12,21
Gender (F/M)	22/38	20/40	25/35
Diagnosis (n)			
Hypertension	15	13	20
Diabetes	25	19	21
KGN	2	10	5
KPN	3	6	4
PCRD	4	5	3
Unknown	11	7	7
Systolic Pressure (mmHg)	144,67 ± 22,80	140,50 ± 28,00	136,50 ± 22,30
Diastolic Pressure (mmHg)	87,58 ± 11,25 [a,b]	82,66 ± 12,73	79,83 ± 10,81
Basal Creatinine (mg/dl)	3,23 ± 0,85 [b,c]	2,00 ± 0,49	2,24 ± 0,67
Basal GFR (ml/dk)	20,66 ± 7,04 [b,c]	35,59 ± 10,21 [d]	31,08 ± 11,15
Basal Calcium (mg/dl)	9,03 ± 0,62 [a,d]	9,25 ± 0,38	9,30 ± 0,48
Basal Phosphor (mg/dl)	4,25 ± 0,67 [b,c]	3,53 ± 0,66	3,62 ± 0,63
Basal Parathormone (pg/ml)	192,50 ± 142,16 [b,c]	119,60 ± 68,46	102, 56 ± 61,94
Basal Hemoglobin (g/dl)	10,86 ± 1,43 [b,c]	12,19 ± 1,63	12,26 ± 1,71
Basal Proteinuria (g/g)	2,98 ± 3,17 [a,b]	1,92 ± 2,29 [d]	0,70 ± 0,68
ACEi/ARB (%)	25,00 [e,f]	46,70	51,70
Eritropoetine (%)	45,00 [b,c]	18,30	13,30
Vitamin D (%)	63,30	68,30	48,30
Atorvastatine (%)	31,70	30,00	16,70
Antiacidose (%)	41,70	35,00	21,70
Follow up period (months)	9,88 ± 6,26	18,06 ± 8,90	16,40 ± 8,64

[a] p<0,05 when compared with Group 2; [b] p<0,001 when compared with Group 3; [c]p<0,001 when compared with Group 2; [d]p<0,05 when compared with Group 3, [e]p<0,01 when compared with Group 3; [f]p<0,01 when compared with Group 2

the control group were determined respectively as 51.9 % (n=40), 39.0 % (n=30) and 9.1 % (n=7). It was observed as a result of the evaluation of the prevalence of all three genotypes between the groups that the result is not statistically significant (p>0.05). It was determined when the allele frequencies for the Fok I gene were examined that the F allele frequency was 69% for Groups 1 and 3 and 71% for Group 2 and the control group (Table 2). Whereas the f allele frequency was determined as 31% for Groups 1 and 3 and as 29% for

Table 2 Genotype and allele frequency for vitamin D receptor FokI gene in chronic renal disease and control group

Genotype frequency	Group 1	Group 2	Group 3	Control
FF	31 (%51,7)	30 (%50,0)	27 (%45,0)	40 (%51,9)
Ff	21 (%35,0)	25 (%41,7)	29 (%48,3)	30 (%39,0)
ff	8 (%13,3)	5 (%8,3)	4 (%6,7)	7 (%9,1)
Allele frequency	Group 1	Group 2	Group 3	Control
F	69%	71%	69%	71 %
f	31%	29%	31%	29%

Group 2 and the control group (Table 2). No statistically significant difference was observed between the groups with regard to allele frequency.

The relationship between the demographic characteristics of the groups, their parametric and nonparametric properties and vitamin D receptors Fok I genotypes have been given in Table 3.

No statistically significant difference was observed between the age average of the patients in all groups and the prevalence of FF, Ff abd ff genotypes. Even though FF genotype is observed more frequently in males in all 3 groups, it was observed that the ff genotype is observed equally in both females and males in Group 1 and more frequently in males at ratios of 1/4 and 0/4 in Groups 2 and 3. However, this difference was not statistically significant (Table 3). No statistically significant relationship could be established between the patient diagnoses and genotype relationship. No statistically significant difference was observed between the groups with regard to ACEi/ARB, EPO, vitamin D, atorvastatine and antiacidose use. A statistically significant relationship could not be observed between the basal and final follow-up biochemical values and VDR FokI genotypes. However, basal creatinine values were measured lower in the ff genotype in all 3 groups. Similarly, basal GFR was determined to be higher in ff genotype individuals in these groups in comparison with the other genotypes. Basal proteinuria levels in all 3 groups were determined to be lower than the ff genotype. However, this difference was not statistically significant (Table 3).

Discussion

Chronic renal disease is becoming more frequent globally (10). Its complications including cardiovascular diseases are starting to become a major public health issue thereby resulting in economic impacts (11).

There was no statistically significant difference in our study between the age average of the groups (Group 1=59,4 years, Group 2=59,7 years and Group 3=63,5 years). No impact was observed on kidney failure progression or RRT requirment. No relationship was determined between advanced age and CRD progression in a study for which age average was calculated as 66,8 years (12). Young age was determined to be related with progression in this study. In another study examining the impacts of age on CRD progression (13), GFR rate of decrease was determined as 2,61 ml/min on average for the elderly population at the end of a monitoring period of 5 years and as an interesting paradox, a greater GFR decrease was detected in the group with normal serum creatinin levels in comparison with the lower Basal GFR. The impact of age and gender on CRD progression was examined in another study (14) in which patients diagnosed with stage 3 CRD were monitored for a period of 10 years and GFR decrease was observed in 73% of these patients. In addition, female gender was determined to be related with a slower decrease in GFR, better patient and kidney survival. While no difference with regard to female/male ratio was determined between the groups in our study even though CRD is observed more frequently in the male population. It is a fact that CRD is observed more in the elderly population in comparison with the young population. In general, accompanying diseases that are observed frequently in the population such as arteriosclerosis, cardiac failure, high blood pressure, diabetes should be taken into consideration as phenomena that may affect CRD progression rather than male gender and physiological process due to age.

The relationship between blood pressure and kidney function is questionable in patients with chronic renal disease (15). The relationship between blood pressure and progression in nondiabetic stage 3 CRD adults was examined during a study in which an average annual GFR decrease of >2.5 ml/min was accepted as fast progression and annual GFR change was determined using kidney function change cystatin C, creatinine and their combination (14). In this study, blood pressure was determined to be related with progression only for the patient group for which cystatin C based or combined equation was used for systolic blood pressure >140 mmHg or diastolic blood pressure 90 >mmHg. No statistically significant relationship was determined in the group for which creatinine based GFR measurement was made. The difference of our study is that fast progression is accepted as an annual average GFR decrease of >4 ml/min and that GFR measurement is made via modification of diet in renal disease (MDRD). The relationship put forth between systolic blood pressure and fast progression and RRT time has been determined in

Table 3 Relationship between the FokI genotypes and the study group characteristics

	Group 1			Group 2			Group 3		
Patient characteristics	**FF**	**Ff**	**ff**	**FF**	**Ff**	**ff**	**FF**	**Ff**	**ff**
n	31	21	8	30	25	5	27	29	4
Age (years)	59,41 ± 2,47	60,76 ± 3,01	56,00 ± 4,88	56,56 ± 2,52	62,04 ± 2,76	67,20 ± 6,17	63,33 ± 2,65	62,51 ± 2,56	71,75 ± 6,90
Gender (F/M)	10/21	8/13	4/4	13/17	6/19	1/4	10/17	15/14	0/4
Diagnosis (n)									
Hypertension	10	4	1	8	5	0	8	9	3
Diabetes	12	10	3	7	10	2	10	10	1
KGN	0	1	1	5	3	2	3	2	0
KPN	1	2	0	5	1	0	1	3	0
PCRD	3	0	1	2	2	1	1	2	0
Unknown	5	4	2	3	4	0	4	3	0
Basal Creatinine (mg/dl)	3,25 ± 0,74	3,29 ± 1,01	2,95 ± 0,87	2,12 ± 0,52	1,90 ± 0,45	1,78 ± 0,33	2,20 ± 0,68	2,30 ± 0,66	2,10 ± 0,87
Basal GFR (ml/dk)	20,03± 6,95	20,97 ± 7,12	22,32 ± 7,81	32,90 ± 11,43	37,90 ± 7,99	40,24 ± 9,66	32,07 ± 10,26	29,41 ± 11,72	36,55 ± 13,29
Basal Calcium (mg/dl)	9,00 ± 0,69	9,19 ± 0,52	8,72 ± 0,48	9,30 ± 0,31	9,20 ± 0,46	9,20± 0,32	9,28± 0,59	9,30± 0,40	9,42 ± 0,29
Basal Phosphor (mg/dl)	4,32 ± 0,76	4,18 ± 0,54	4,15 ± 0,66	3,69 ± 0,76	3,37 ± 0,61	3,36 ± 0,25	3,64 ± 0,72	3,64 ± 0,51	3,40 ± 0,77
Basal Parathormone (pg/ml)	170,19 ± 122,01	189,62 ± 131,13	286,50 ± 212,30	101,43 ± 54,22	138,08 ± 76,67	136,20 ± 87,14	106,33 ± 56,89	102,10 ± 69,96	80,50 ± 30,98
Basal Hemoglobin (g/dl)	10,80 ± 1,44	10,95± 1,08	10,86 ± 2,24	11,77 ± 1,67	12,66 ± 1,51	12,40 ± 1,66	12,49 ± 1,50	11,91± 1,91	13,25 ± 0,90
Basal proteinuria (g/g)	3,36 ± 3,52	2,60 ± 2,81	2,53 ± 2,80	2,53 ± 2,68	1,38 ± 1,71	0,94± 1,32	0,71 ± 0,69	0,75 ± 0,71	0,20 ± 0,20
Final Creatinine (mg/dl)	4,95 ± 1,40	5,40 ± 1,55	5,27 ± 0,91	3,27 ± 1,54	2,55± 0,76	2,24 ± 0,64	2,00 ± 0,72	2,19 ± 0,79	2,25 ± 1,38
Final GFR (ml/dk)	11,57 ± 3,96	10,64 ± 3,51	11,17 ± 2,64	22,68 ± 11,04	28,08 ± 8,57	31,60 ± 10,73	36,79 ± 12,90	31,94 ± 12,76	36,75 ± 18,78
Final Calcium (mg/dl)	8,93 ± 0,50	8,84 ± 0,77	9,15± 1,04	9,40± 0,91	9,28 ± 0,39	9,78± 1,11	9,36 ± 0,48	9,31 ± 0,49	9,65 ± 0,67
Final Phosphor (mg/dl)	5,01 ± 0,15	4,99 ± 0,18	4,58 ± 0,30	4,31 ± 0,15	3,60 ± 0,17	3,94 ± 0,38	3,63 ± 0,16	3,66 ± 0,15	2,82 ± 0,42
Final Parathormone (pg/ml)	183,04 ± 20,09	218,28 ± 24,42	183,50 ± 39,56	116,60 ± 20,43	115,52 ± 22,38	120,20 ± 50,04	113,29 ± 21,53	115,17 ± 20,78	118,75 ± 55,95
Final Hemoglobin (g/dl)	10,87 ± 1,34	10,20 ± 1,31	11,10 ± 1,38	11,44 ± 1,88	12,51 ± 1,55	12,16 ± 1,24	12,99± 1,46	12,15 ± 1,38	13,32 ± 2,23
Final Proteinuria (g/g)	3,79 ± 3,68	2,95 ± 2,96	5,88 ± 6,76	1,97 ± 2,31	1,57 ± 2,68	0,52 ± 0,30	1,28 ± 1,79	0,93 ± 1,10	0,35 ± 0,50
ACEi/ARB (n)	7	6	2	12	13	3	15	14	2
Eritropoetin (n)	14	10	3	9	2	0	3	5	0
Vitamin D (n)	17	15	6	20	19	2	14	13	2
Atorvastatine (n)	13	3	3	6	10	2	6	4	0
Antiacidose (n)	10	12	3	13	7	1	4	8	1

our study (12). On the other hand, similar results have been obtained in another study in which a similar method was used with our study during which 211 adult individuals with stage 3-5 CRD diagnosis with an average monitoring period of 56,6 months were retrospectively reviewed (16). These researchers could not determine a statistically significant relationship between the initial systolic and diastolic blood pressures and predialytic CRD progression. Similarly, the non-existent relationship between CRD progression and the blood pressure level at the time of application was also put forth in an observational study carried out on 1094 Afro-American patients (17) and another study carried out on patients with polycystic renal disease (18).

In addition to the expected contribution of age and CRD stage, it has been set forth that there is a correlation between the progression of renal disease and proteinuria and that the progress is slowed down with the use of angiotensin converting enzyme inhibitor (ACEi) and angiotensin receptor blockers (ARB) (19-21). Our results are in compliance with the results of these studies. These researchers have examined the relationship between proteinuria and ACEi/ARB use on predialysis monitored patients and decrease in renal functions and/or RRT onset. A total of 547 stage 4-5 CRD predialysis patients were monitored for a period of about 7 years in the study as a result of which a decrease of 0,35 ml/min/1.73 m2/month on average was observed in renal functions in patients with light proteinuria (beteen >0,3 and ≤1,0 g/24 h). When patients undergoing RRT were compared with patients without proteinuria (≤0,3 g/24 hour) a higher decrease in renal functions was observed. When the level of proteinuria was taken into consideration (between >1,0 and ≤3,0 arası, >3,0 and ≤6,0 and >6,0 g/24 hour), it was observed that early RRT requirement was correlated with increasing proteinuria and increased progression. On the other hand, a lower RRT start rate was determined when patients using ACEi/ARB at the start (n=16) or during follow-up (n=133) were compared with the patients who do not use ACEi/ARB (n=152). In conclusion, they were of the opinion that there is no proof regarding the harm of using ACEi/ARB in predialysis monitored patients and that proteinuria may be used as an indicator of CRD progression risk. It was determined in another study (21) that the rates of ACEi/ARB use were 31%, 46% and 51% respectively for patient groups with GFR <15 ml/min, GFR 15-24 ml/min and GFR 25-29 ml/min. These findings support our results. In addition, findings indicating a relationship between proteinuria above 1 g/g and fast progression and RRT requirement are also in accordance with the findings of our study.

The issue on the relationship between statin use and low mortality as well as its impact on decreasing RRT requirement and renal failure progression is a matter of debate (22-25). No statistically significant difference was determined in our study between statin use among groups. The reason for the conflicting results between renal progression and statin use may be the fact that majority of the data have been acquired from studies on cardiovascular primary endpoints.

Metabolic acidosis is traditionally defined as a decrease in blood pH related with a decrease in serum bicarbonate (HCO_3) concentration and is related with progressive CRD (26). There is a correlation between HCO_3 decrease due to nephron loss and impairment in ammoniac metabolism and GFR decrease. This may be related with the progression of metabolic acidose renal disease and it has been put forth that CRD progression may be prevented if it is improved (27). Low and high serum bicarbonate levels were determined in 13.9 % (n=5796) and 1.6 % (n=652) of the patients respectively during a study carried out on 41749 patient individuals (28) and a statistically significant relationship was determined between low serum bicarbonate and mortality related with all causes after the related co-variables were adjusted. Even though this relationship is not statistically significant between stage 4 CRD and diabetes patients, the importance of the relationship between mortality and decrease in bicarbonate level from normal to low levels has been indicated. High serum bicarbonate levels were determined to be related with mortality regardless of renal function level. However, the fact that serum bicarbonate levels have not been taken into consideration in our study was a limiting factor in establishing this relationship. The fact that sodium bicarbonate use is higher in the Group with RRT requirement (41,70 %) and the Group with progression (35 %) in comparison with the stable GFR group (21,70 %) indirectly indicates the probable contribution of acidose in renal failure process even though the difference is not statistically significant.

It has been reported that bringing hemoglobin levels to normal for patients with chronic renal disease prevents tubular damage and interstitial fibrosis development by improving oxygen flow to the kidneys thereby reducing renal disease progression. In addition, it has also been set forth that the use of EPO prevents oxidative stress and apoptosis and may have direct protective impacts on tubular cells (29, 30). Low hemoglobin levels in the group with RRT requirement in our study leads us to think that anemia is observed more frequently in this group with further decrease in GFR and that it may be effective in progression to end-stage renal disease (ESRD).

Accumulation and combination of genetic and environmental factors may play a role on the onset and progression of ESRD in CRD which is accepted as a multifactorial disease. For example, there may be genetic impacts on the development of hyperparathyroidism secondary to renal failure and VDR gene from among these genes may play a role in progression. Vitamin D plays a role in the regulation of independent biological processes such as the endochrine system, bone metabolism, immune response from birth, cell proliferation and differentiaton (5). Vitamin D response takes place with VDR function. VDR gene is present in many tissues and its activation may modulate more than one target gene expression. Various polymorphisms have been determined in the VDR gene and their functional importance along with potential impacts on disease predisposition have been examined (31, 32). Significant allele variations of the VDR gene in different populations have attracted attention in these studies. FokI polymorphism is one of these allele variations.

Individual allele frequency varies between different ethnic or geographical populations. F allele is observed more frequently than f allele in many societies including our own. For instance, F and f allele distribution was observed as 71,5 % and 28,5 % in a study carried out on healthy population in Northern India [32] which was in accordance with the results of our study. When F/f allele frequency is examined, it can be observed that F allele is more frequent in Finland (60/40), England (69/31), Australia (61/39), Japan (68/32), Taiwan (61/39), White Massachusets (59/41) and Black Pensylvania (78/22) [33]. Allele frequencies that are similar to our results (73 % F and 27 % f) were obtained as a result of a previous study (34) carried out on healthy Turkish population. FokI polymorphism differs among different populations with regard to homozygote and heterozygote mutations. For example, even though the FF genotype ratios are higher in England and Black Pensylvania in accordance with our results (48 % and 63 % respectively), Ff genotype was observed at higher ratios in Northern India (49 %), Finlan (58 %), Australia (48 %), Japan (51 %), Taiwan (49 %) and White Massacusets (45 %) societies. Whereas the ff genotype was determined as the genotype with the lowest frequency even though it is observed at various rates in all societies. However, its ratios were determined more in Finland (14 %), Australia (15 %) and White Massachusetts (18 %) populations in comparison with other societies (34).

The number of studies on the impact of FokI polymorphism on CRD progression is limited (35). The impact of VDR gene ApaI, TaqI, FokI and BsmI polymorphisms has been examined on 258 ESRD patients and 569 healthy control group in the Northern India population as a result of which a statistically significant difference was observed in the FokI ff ($p=0.001$) genotype frequency (35). It was put forth as a result of the haplotype analyses that individuals with a/t/F/b haplotypes are prone to 11,0 times greater risk. In conclusion, they have asserted that VDR gene FokI polymorphism is related with ESRD (35). Nevertheless, a relationship could not be determined in our study between FokI polymorphism and renal progression and RRT requirement. A statistically significant difference could not be observed between the healthy control group and patient groups with regard to FokI gene polymorphism. However, it was determined in all 3 groups that the basal creatinine values were lower in the ff genotype and thus the Basal GFR levels were higher. Even though the difference is not statistically significant, these results have brought about the opinion regarding the need for future studies related with the positive impact of ff genotype on renal progression.

Similar results have been obtained in another study as well (36). These researchers have examined the relationship between FokI polymorphism and 1,25 OHD vitamin levels and CRD stages during a study carried out with 410 type 2 diabetic CRD patients as a result of which no relationship was determined between FokI

polymorphism and CRD which is in accordance with our results. Moreover, they observed an interaction between ff genotype and 1,25 OHD vitamin (p=0,008). They determined that the negative relationship between 1,25 OHD and CRD stages was repressed more in FokI ff genotype rather than FokI FF genotype.

The relationship between VDR start codon polymorphism (FokI) and PTH levels, calcidiol and calcium was examined in another study carried out on 64 Spanish CRD patients [37] as a result of which the genotype frequencies were determined in the patient population as 54,7 % FF, 28,1 % Ff and 17,2 % ff; whereas the values for the healthy control population were determined as 46,7 % FF, 43,3 % Ff, 10 % ff. The difference between the patient and control groups was determined to be statistically significant ($p<0.01$). Serum PTH levels in the FF genotype group patient population (159,77 +/- 25,69 pg/ml) to be higher at a statistically significant level in comparison with both Ff and ff groups (106.67 +/- 19,07 and 77.55 +/- 15,85 pg/ml, respectively, $p <0.05$). However, no statistically significant difference was observed between the genotypes with regard to calcidiol or calcium levels (37). Contrary to this, a statistically significant relationship was determined in our study between PTH levels and FokI polymorphism. No statistically significant difference was observed between the calcium levels. The reason for this may be due to the fact that a certain polymorphism most likely does not manifest itself among ethnic groups even though ethnic differences are observed between VDR gene polymorphisms. Because there is the opinion that the physiological role of vitamin D endocrine system is the same in all ethnic groups (5). These results seem insufficient in determining the impact of polymorphism on progression and PTH response in CRD patients. In addition, vitamin D deficiency and vitamin D resistance in ESRD depends on many different factors. Therefore, there is a need for more comprehensive randomized controlled studies depending on vitamin D plasma level measurement or vitamin D treatment status.

On the other hand, ethnic variations of the VDR gene may yield beneficial results in genetic studies due to the fact that VDR may play a role in the progression of various chronic inflammatory and degenerative diseases and due to its polymorphic content. Significant differences in FokI allele frequency will be beneficial for establishing a bond between the individuals in different societies. These studies may provide a foresight for determining the sensitivity towards certain diseases and the clinical management of patients in the long run.

Funding
This study was not funded by any organization.

Compliance with ethical standards
Conflict of interest
The authors declare no conflict of interest.

Ethical approval
All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

References

1. liçin G, Ünal S, Biberoğlu K, Akalın S, Süleymanlar G (2003) Temel Çocuk Hastalıkları. 3.baskı, Ankara, Güneş Kitabevi; 769-777.
2. Coresh J, Selvin E, Stevens LA (2007) Prevalence of chronic kidney disease in the United States. JAMA 298: 2038-2047.
3. Stevens LA, Li S, Wang C (2010) Prevalence of CKD and comorbid illness in elderly patients in the United States: results from the Kidney Early Evaluation Program (KEEP). Am J Kidney Dis 55: 23-33.
4. Ekmekçi A, Konaç E, Ülke Önen H (2008) Gen polimorfizmi ve kansere yatkınlık. Marmara Medical Journal 21: 282-295.
5. Kostner K, Denzer N, Muller CSL, Klein R, Tilgen W, Reichrath J (2009) The Relevance of Vitamin D Receptor (VDR) Gene Polymorphisms for Cancer: A Review of the Literature. Anticancer Research 29: 3511-3536.
6. Uitterlinden AG, Fang Y, Van Meurs JB, Pols HA, Van Leeuwen JP (2004) Genetics and biology of vitamin D receptor polymorphisms. Gene 338: 143–156.
7. Gross C, Eccleshall TR, Mallory PJ, Villa ML, Marcus R, Feldman D (1996) The presence of a polymorphism at the translation initiation site of the vitamin D receptor gene is associated with low bone mineral density in postmenopausal Mexican American women. J Bone Miner Res 11: 1850-1855.
8. Ntais C, Polycarpou A, Ioannidis JPA (2003) Vitamin D receptor gene polymorphisms and risk of prostate cancer: A meta-analysis. Cancer Epid, Biomarkers & Prev 12: 1395-1402.
9. Sel SK, Kasap H (2011) Osteoporoz ve İlişkili Genler: VDR, ESR ve COL1A1. Ar-iv 20: 246.
10. Zhang QL, Rothenbacher D (2008) Prevalence of chronic kidney disease in population-based studies: systematic review. BMC Public Health 8: 117.
11. Khan S, Amedia CA (2008) Economic burden of chronic kidney disease. J Eval Clin Pract 14: 422–434.

12. Levin A, Djurdjev O, Beaulieu M, Lee E (2008) Variability and risk factors for kidney disease progression and death following attainment of stage 4 CKD in a referred cohort. American Journal of Kidney Diseases 52: 661-671.
13. Heras M, Fernandez-Reyes MJ, Sanchez R, Guerrero MT, Molina A, Rodríguez MA (2012) Elderly patients with chronic kidney disease: outcomes after 5 years of follow-up. Nefrologia 32: 300-305.
14. Eriksen BO, Ingebretsen OC (2006) The progression of chronic kidney disease: A 10-year population-based study of the effects of gender and age. Kidney International 69: 375–382.
15. Bloomfield GS, Yi SS, Astor BC, Kramer H, Shea S, Shlipak MG, Post WS (2013) Blood pressure and chronic kidney disease progression in a multi-racial cohort: the Multi-Ethnic Study of Atherosclerosis. J Hum Hypertens 14.
16. Pereira AC, Carminatti M, Fernandes NM, Tirapani Ldos S, Faria Rde S, Grincenkov FR, Magacho EJ, Carmo WB, Abrita R, Bastos MG (2012) Association between laboratory and clinical risk factors and progression of the predialytic chronic kidney disease. J Bras Nefrol 34: 68-75.
17. Luke RG (1999) Hypertensive nephrosclerosis: pathogenesis and prevalence. Essential hypertension is an important cause of end-stage renal disease. Nephrol Dial Transplant 14: 2271-2278.
18. Van Dijk MA, Breuning MH, Duiser R (2003) No effect of enalapril on progression in autosomal dominant polycystic kidney disease. Nephrol Dial Transplant 18: 2314-2320.
19. Lea J, Cheek D, Thornley-Brown D (2008) Metabolic syndrome, proteinuria, and the risk of progressive CKD in hypertensive African Americans. Am J Kidney Dis 51: 732-740.
20. Hou FF, Xie D, Zhang X (2007) Renoprotection of optimal antiproteinuric doses (ROAD). Study: a randomized controlled study of benazepril and losartan in chronic renal insufficiency. J Am Soc Nephrol 18: 1889-1898.
21. De Goeij MC, Liem M, De Jager DJ, Voormolen N, Sijpkens YW, Rotmans JI (2012) Proteinuria as a risk marker for the progression of chronic kidney disease in patients on predialysis care and the role of angiotensin-converting enzyme inhibitor/angiotensin II receptor blocker treatment. Nephron Clin Pract 121: 73-82.
22. Huskey J, Lindenfeld J, Cook T (2009) Effect of simvastatin on kidney function loss in patients with coronary heart disease: findings from the Scandinavian Simvastatin Survival Study (4S). Atherosclerosis 205: 202-206.
23. Rahman M, Baimbridge C, Davis BR (2008) Progression of kidney disease in moderately hypercholesterolemic, hypertensive patients randomized to pravastatin versus usual care: a report from the antihypertensive and lipid-lowering treatment to prevent heart attack trial (ALLHAT). Am J Kidney Dis 52: 412-424.
24. Nakamura T, Ushiyama C, Hirokawa K, Osada S, Shimada N, Koide H (2001) Effect of cerivastatin on urinary albumin excretion and plasma endothelin-1 concentrations in type 2 diabetes patients with microalbuminuria and dyslipidemia. Am J Nephrol 21: 449–454.
25. Strippoli GF, Navaneethan SD, Johnson DW, Perkovic V, Pellegrini F, Nicolucci A, Craig JC (2008) Effects of statins in patients with chronic kidney disease: meta-analysis and meta-regression of randomised controlled trials. BMJ 336: 645–651.
26. Kraut JA, Kurtz I (2005) Metabolic acidosis of CKD; diagnosis,clinical characteristics and treatment. Am J Kidney Dis 45: 978-993.
27. Kraut JA, Madias NE (2011) Consequences and therapy of the metabolic acidosis of chronic kidney disease. Pediatr Nephrol 26: 19-28.
28. Navaneethan SD, Schold JD, Arrigain S, Jolly SE, Wehbe E, Raina R, Simon JF, Srinivas TR, Jain A, Schreiber MJ, Nally JV (2011) Serum bicarbonate and mortality in stage 3 and stage 4 chronic kidney disease. Clin J Am Soc Nephrol 6: 2395-402.
29. Sharples EJ, Patel N, Brown P (2004) Erythropoietin protects the kidney against the injury and dysfunction caused by ischemiareperfusion. J Am Soc Nephrol 15: 2115-2124.
30. Gouva C, Nikolopoulos P, Ioannidis JP, Siamopoulos KC (2004) Treating anemia early in renal failure patients slows the decline of renal function: a randomized controlled trial. Kidney Int. 66: 753-760.
31. Zmuda JM, Cauley JA, Ferrell RE (2000) Molecular epidemiological of Vitamin D receptor variants. Epidemiol. Rev 22: 203-17.
32. Bid HK, Mittal RD (2003) Study of vitamin-D receptor (VDR) gene start codon polymorphism (Fok I) in healthy individuals from North India. J Hum Genet 9: 51-54.
33. Hemant KB, Dhruva KM, Rama DM (2013) Vitamin-D receptor (VDR) gene (Fok-I, Taq-I & Apa-I) polymorphisms in healthy individuals from north Indian population. Asian Pacific J Cancer Prev 6; 147-152
34. Dayangaç D, Özaydın E, Gerçeker FÖ, Co kun T, Yurter HE (2002) Sa lıklı Türk populasyonunda Vitamin D reseptör (VDR) gen analizi. Türk Biyokimya Dergisi 27: 1.
35. Tripathi G, Sharma R, Sharma RK, Gupta SK, Sankhwar SN, Agrawal S (2010) Vitamin D receptor genetic variants among patients with end-stage renal disease. Ren Fail 32: 969-977.
36. Yokoyama K, Nakashima A, Urashima M, Suga H, Mimura T, Kimura Y, Kanazawa Y, Yokota T (2012) Interactions between serum vitamin D levels and vitamin D receptor gene FokI polymorphisms for renal function in patients with type 2 diabetes. PLoS One 7: 51171.
37. Vigo Gago E, Cadarso-Suárez C, Perez-Fernandez R, Romero Burgos R, Devesa Mugica J, Segura Iglesias C (2005) Association between vitamin D receptor FokI. polymorphism and serum parathyroid hormone level in patients with chronic renal failure. J Endocrinol Invest 28: 117-121.

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