Association of the BsmI, ApaI, TaqI, Tru9I and FokI Polymorphisms of the Vitamin D Receptor Gene with Nephrolithiasis in the Turkish Population

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Purpose: To analyze the relationship between nephrolithiasis and vitamin D receptor (VDR) gene BsmI (rs1544410), ApaI (rs7975232), TaqI (rs731236), Tru9I (rs757343) and FokI (rs2228570) polymorphisms in a study group from the Turkish population.

Materials and Methods: Ninety-eight patients with calcium oxalate kidney stones and 70 controls were enrolled in this study. Five polymorphisms of the VDR gene were studied using the Polymerase Chain Reaction Restriction Fragment Length Polymorphism (PCR-RFLP) method.

Results: For all polymorphisms, genotype frequencies were in line with Hardy-Weinberg equilibrium in the patients and controls. For the BsmI polymorphism, allele frequency distribution was found to differ significantly between the patients and the controls ($P < .05$). The "B" allele was found to increase the risk of nephrolithiasis by approximately 1.5-fold (odds ratio = 1.55, 95% confidence interval: 1.00-2.40; $P = .048$). However, we did not find any statistically significant differences in the allele and genotype frequencies for the ApaI, TaqI, Tru9I and FokI polymorphisms. Proportionally, the "BAt" and "baT" haplotypes were more common than other haplotypes in the cases and controls, respectively. For the haplotypes of the BsmI and TaqI polymorphisms, the "bT" haplotype frequency was found to be common in both the patients and the controls. However, we did not find statistically significant differences between the cases and the controls for either the BsmI / ApaI / TaqI or the BsmI/TaqI haplotypes. Moreover, no relationship was identified between family history and development of stone disease.

Conclusion: The "B" allele of the BsmI polymorphism of the VDR gene may increase stone development risk. Further investigations are needed to improve our knowledge regarding the genetic factors affecting urinary stone development.

Keywords: nephrolithiasis; genetics; mutation; humans; vitamin d3 24-hydroxylase/genetics; metabolism; hypercalcemia; hypercalciuria; nephrocalcinosis.

INTRODUCTION

Nephrolithiasis is a common multifactorial disease that is influenced by both environmental and genetic factors.[1] Several studies have reported an increase in its morbidity rate in recent years.[2] Moreover, it has a recurrence rate of approximately 50% within 10 years. Most stones (up to 75%) are composed of calcium oxalate (CaOx) crystals, and their formation occurs in a complex manner.[3]

With regard to understanding the genetic basis of the disease, several single nucleotide polymorphisms (SNPs) have been analyzed to determine the risk of stone formation in different populations. To date, SNPs found in osteopontin (SPP1),[4] calcium sensing receptor (CaSR), matrix-gla protein (MGP), urokinase (PLAU) (recently reviewed by Arcidiacono and colleagues),[5] E-cadherin (CHD1),[6] calcitonin receptor (CALCR),[7] transient receptor potential vanilloid member 5 (TRPV5),[8] Fetuin-A[9] and vitamin D receptor (VDR)[10-23] genes have been analyzed, and some of them are related to nephrolithiasis development.

The VDR gene encodes a nuclear receptor for the active form of vitamin D, 1,25-dihydroxy vitamin D3 [$1,25(OH)_2D_3$]. After it binds to its response element on DNA, it regulates hundreds of genes with different functions, including calcium homeostasis.[24]

The VDR gene has several polymorphisms, and some of them have been associated with urinary stone formation in some populations.[10-23] Whereas the FokI (rs2228570, c.2T > C, p.Met1Arg) polymorphism

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is located in the second exon of the VDR gene, the BsmI (rs1544410, 1024+283G > A), ApaI (rs7975232, c.1025-49G > T), TaqI (rs731236, c.1056T > C, p.Ile352Ile) and Tru9I (rs757343, c.1024+443G > A) polymorphisms are found at the 3′ end of the gene.[25] The FokI (rs2228570, c.2T > C, p.Met1Arg) polymorphism changes the translation start codon and causes the production of two different VDR protein variants. The first one is a long variant and is coded by the T allele or the "f" allele. The second short variant is shortened by three amino acids and is coded by the C-allele or the "F" allele.[25] Compared with the long VDR form, the short form has greater transcriptional activation capability.[26] The BsmI (rs1544410, 1024+283G > A), ApaI (rs7975232, c.1025-49G>T) and Tru9I (rs757343, c.1024+443G > A) polymorphisms are located in the 8th intron of the gene,[25] whereas the TaqI (rs731236, c.1056T > C, p.Ile352Ile) polymorphism is a synonymous variation at codon 352 in exon 9 of the gene, and this T > C alteration does not change the amino acid sequence (p.Ile352Ile).[27] The BsmI, ApaI, Tru9I and TaqI polymorphisms are located at the 3′ end of the gene and are near the regulatory 3' untranslated region (3'-UTR) of mRNA. Thus, when the BsmI, ApaI, and TaqI polymorphisms are found in specific haplotypes, they have been shown to affect VDR mRNA stability and the rate of transcription.[19] For example, in green monkey kidney cells, the "BAt" haplotype of the Bsm / Apa / Taq polymorphisms was shown to increase VDR expression compared with the "baT" haplotype.[27] A relationship between the bb genotype of the BsmI polymorphism and higher urinary calcium extraction and a link between the T allele of the TaqI polymorphism and hypocitraturia were shown in the literature. However, there are conflicting data regarding the relationship between the FokI and ApaI polymorphisms and urinary stone formation mechanisms.[28]

There is a lack of knowledge in the literature regarding the association of the BsmI, ApaI, TaqI, Tru9I and FokI polymorphisms of the VDR gene with nephrolithiasis in the Turkish population. Thus, in this study, we aimed to investigate the possible relationship between development of nephrolithiasis and five common polymorphisms [BsmI (rs1544410), ApaI (rs7975232), TaqI (rs731236), Tru9I (rs757343) and FokI (rs2228570)] of the VDR gene in patients and control subjects from the Turkish population. Moreover, a haplotype analysis was also performed for the BsmI, ApaI and TaqI polymorphisms.

MATERIALS AND METHODS

Study Population

Between March 2006 and March 2008, patients with urinary calcium oxalate stones who were treated at the Istanbul Sisli Etfal Research and Training Hospital and Erzurum Numune Hospital were enrolled in the study. The study population was composed of 98 patients with CaOx nephrolithiasis (65 male and 33 female) who were initially diagnosed by computerized tomography (CT) scan. Fifty-two (53%) cases out of 98 had familial stone history (maternal and/or paternal urolithiasis history), and 33 of them were male. For the control group, seventy individuals (52 male and 18 female) without family history or clinical signs of urinary stone disease (assessed via urine testing, plain abdominal radiography, or ultrasonography) were included in the study. All cases and controls were matched according to age and gender. Eleven of 98 patients had recurrent stone disease. After treatment with extracorporeal shock wave lithotripsy (SWL) or surgery, stone samples were collected, and stone analysis was performed using an X-ray diffraction (XRD) machine. XRD analysis confirmed that they contained calcium oxalate crystals. Patients with Ca oxalate stones were included in the study. Patients with abnormal levels of serum creatinine, calcium, phosphate, uric acid, liver enzymes, sodium, potassium, chloride and $1,25(OH)_2D_3$, thyroid-stimulating hormone, free thyroxin, free triiodothyronine, and parathyroid hormone were excluded from the study. Furthermore, patients diagnosed with hypercalcemia, renal tubular acidosis, hyperuricemia, gout, hyperparathyroidism, urinary tract infection, renal failure and hyperthyroidism were also excluded from the study. The last group of exclusion criteria included patients who take drugs that effect calcium and hormone metabolism, such as diuretics, calcium and vitamin D supplements, and anti-diabetic and anti-hypertensive agents.

Informed consent was obtained from all patients and healthy subjects in accordance with the Helsinki Declaration (revised 2001), and the study protocol was approved by the local ethics committee (approval number: IRB 2007 / 88-685)

Analysis of VDR Gene Polymorphisms

Blood samples were collected in ethylenediaminetetraacetic acid (EDTA) containing tubes, and the genomic DNA of the study subjects was isolated with a High Pure polymerase chain reaction (PCR) Template Preparation Kit (Roche, Mannheim, Germany) according to the manufacturer's protocol. The concentrations and purity of the samples were checked spectrophotometrically. The detection of the 5 polymorphisms [BsmI

Table 1. Primers, annealing temperatures, product sizes, restriction enzymes, and allele sizes used for vitamin D receptor gene genotyping.

Polymorphism	Chr 12: position	Methodical Nomenclature	Primer Sequences	Annealing Temperature (°C)	Product Size (bp)	Restriction Enzyme	Alleles
BsmI[29]	47846052	Intronic	F: 5′-CAACCAAGACTACAAGTACCGCGTCAGTGA-3′	63	825	BsmI	G (b): 650+175
(rs1544410)	(forward strand)	(1024+283 G>A)	R: 5′-AACCAGCGGGAAGAGGTCAAGGG-3′				A (B): 850
ApaI[29]	47871419	Intronic	F: 5′-CAGAGCATGGACAGGGAGCAA-3′	60	722	ApaI	T (A): 722
(rs7975232)	(forward strand)	(c.1025-49 G > T)	R: 5′-GCAACTCCTCATGGCTGAGGTCTC-3′	60			G (a): 509+213
TaqI[29]	47844974	Synonymous	F: 5′-CAACCAAGACTACAAGTACCGCGTCAGTGA-3′	63	2000	TaqI	T (T): 2000
(rs731236)	(forward strand)	(p.Ile352Ile)	R: 5′-CACTTCGAGCACAAGGGGCGTTAGC-3′				C (t): 1800+200
		c.1056 T > C					
Tru9I[28]	47845892	Intronic	F: 5′-TGTATTGGTCCAGCTTGCTCT-3′	63	252	Tru9I	A (u): 193+59
(rs757343)	(forward strand)	c.1024+443G > A	R: 5′-CAGGGTTTCTCCATGTTGGT-3′				G (U): 252
FokI[30]	47879112	Start lost	F: 5′-AGCTGGCCCTGGCACTGACTCTGCTCT-3′	63	265	FokI	T (f): 196+69
(rs2228570)	(forward strand)	c.2T > C	R: 5′-ATGGAAACACCTTGCTTCTTCTCCCTC-3′				(p.Met1Arg)

(rs1544410), ApaI (rs7975232), TaqI (rs731236), Tru9I (rs757343) and FokI (rs2228570)] of the VDR gene was carried out using the conventional Polymerase Chain Reaction Restriction Fragment Length Polymorphism (PCR-RFLP) method.[29-31] Each amplification reaction was performed in a 25 µL final volume consisting of 1 U/µL unit Taq DNA polymerase, each primer at a concentration of 10 pmol/µL, a 100 ng DNA sample, 100 µM dNTP, and 2 mM MgCl2. Primer sequences, annealing temperatures, restriction endonucleases and allele sizes are provided in **Table 1**. The PCR and restriction enzyme products were electrophoresed in a 2.0% agarose gel and then stained with ethidium bromide. For all studied polymorphisms, agarose gel electrophoresis pictures demonstrating alleles and genotypes are provided in **Figure 1** A-E.

DNA Sequencing

For all polymorphisms, homozygote and heterozygote samples identified via PCR-RFLP analysis were confirmed by DNA sequencing in the laboratories of Iontek (Istanbul, Turkey). The sequences obtained were analyzed using Clustal W (version 1.83) alignment software and compared with the RFLP results. All sequenced samples were in agreement with the RFLP data.

Statistical Analysis

Descriptive statistics with a normal distribution were presented as the mean ± standard deviation, and nominal variables were presented as numbers of cases and percentages (%). Distributions of the groups were evaluated with the Kolmogorov–Smirnov and Shapiro-Wilk normality tests. The significances of the differences between the two groups were evaluated with Student's t-test for the means. Categorical variables were evaluated using Pearson's Chi-square or Fisher's exact test. The distributions of the genotype and allele frequencies between the groups and their relationship to CaOx nephrolithiasis were compared with a Chi-square (χ^2) test. Hardy–Weinberg equilibrium (HWE) was also tested using the χ^2 test based on observed and expected

Table 2. Characteristics of the study groups.

Variables	Patients (n = 98)	Controls (n = 70)	*P* Value
Age, years, mean ± SD	47.2 ± 16.3	42.6 ± 13.5	.536
Gender, no (%)			
Male	65 (66.3)	52 (74.3)	.349
Female	33 (33.7)	18 (25.7)	.349
BMI, kg/m^2, mean ± SD	24.9 ± 3.7	24.1 ± 3.1	.552
Smoking, no (%)	40 (40.8)	33 (47.1)	.511
Family history, no (%)	52 (53)	-----	-----

Abbreviations: BMI, body mass index; SD, standard deviation.

Table 3. Genotype and allele frequency of the five vitamin D receptor gene polymorphisms in the patients and controls and the results of the Hardy-Weinberg Equilibrium tests.*

Variables	Patients (n = 98)	HWE *P* Value	Adjusted HWE**P* Value	Controls (n = 70)	HWE *P* Value	Adjusted HWE** *P* Value
BsmI		.568	.673		.781	.911
BB	29 (29.6)			13 (18.6)		
Bb	46 (46.9)			33 (47.1)		
bb	23 (23.5)			24 (34.3)		
BB+ Bb	75 (76.5)			46 (65.7)		
Allele frequency						
B	104 (53.1)			59 (42.1)		
b	92 (46.9)			81 (57.9)		
ApaI		.271	.351		.834	.948
AA	43 (43.9)			26 (37.1)		
Aa	40 (40.8)			34 (48.6)		
aa	15 (15.3)			10 (14.3)		
Aa+aa	55 (56.1)			44 (62.9)		
Allele frequency						
A	126 (64.3)			86 (61.4)		
a	70 (35.7)			54 (38.6)		
TaqI		.443	.541		.454	.582
TT	35 (35.7)			31 (44.3)		
Tt	44 (44.9)			29 (41.4)		
tt	19 (19.4)			10 (14.3)		
Tt+tt	63 (64.3)			39 (55.7)		
Allele frequency						
T	114 (58.2)			91 (65.0)		
t	82 (41.8)			49 (35.0)		
Tru9I		.223	.290		.176	.249
UU	18 (18.4)			6 (8.6)		
Uu	41 (41.8)			37 (52.9)		
uu	39 (39.8)			27 (38.6)		
Uu+uu	80 (81.6)			64 (91.4)		
Allele frequency						
U	77 (39.3)			49 (35.0)		
u	119 (60.7)			91 (65.0)		
FokI		.305	.399		.494	.658
FF	48 (49)			39 (55.7)		
Ff	38 (38.8)			25 (35.7)		
ff	12 (12.2)			6 (8.6)		
Ff+ff	50 (51.0)			31 (44.3)		
Allele frequency						
F	134 (68.4)			103 (73.6)		
f	62 (31.6)			37 (26.4)		

Abbreviation: HWE, Hardy-Weinberg Equilibrium.
* Data are presented as no (%)
genotype numbers with the 'Hardy Weinberg' package
$ Chi-Square *P* value with continuity correction.

Table 4. Genotype and allele frequency of the five vitamin D receptor gene polymorphisms in the patients and controls.*

Variables	Patients (n = 98)	Controls (n = 70)	*P* Value	OR (95% CI)	*P* Value
BsmI					
BB	29 (29.6)	13 (18.6)	.158	2.33 (0.98-5.55)	.055
Bb	46 (46.9)	33 (47.1)		1.45 (0.70-3.01)	.311
bb	23 (23.5)	24 (34.3)		1.00**	
BB+ Bb	75 (76.5)	46 (65.7)		1.70 (0.86-3.36)	.124
Allele frequency					
B	104 (53.1)	59 (42.1)	.048	1.55 (1.00-2.40)	.048
b	92 (46.9)	81 (57.9)		1.00**	
ApaI					
AA	43 (43.9)	26 (37.1)	.595	1.00**	
Aa	40 (40.8)	34 (48.6)		0.71 (0.36-1.39)	.317
aa	15 (15.3)	10 (14.3)		0.91 (0.35-2.31)	.838
Aa+aa	55 (56.1)	44 (62.9)		0.77 (0.40-1.41)	.382
Allele frequency					
A	126 (64.3)	86 (61.4)		1.00**	
a	70 (35.7)	54 (38.6)		0.88 (0.56-1.39)	.593
TaqI					
TT	35 (35.7)	31 (44.3)	.474	1.00**	
Tt	44 (44.9)	29 (41.4)		1.34 (0.68-2.63)	.389
tt	19 (19.4)	10 (14.3)		1.68 (0.68-4.16)	.258
Tt+tt	63 (64.3)	39 (55.7)		1.43 (0.76-2.68)	.262
Allele frequency					
T	114 (58.2)	91 (65.0)		1.00**	
t	82 (41.8)	49 (35.0)		1.34 (0.85-2.09)	.205
Tru9I					
UU	18 (18.4)	6 (8.6)	.148	2.08 (0.73-5.91)	.166
Uu	41 (41.8)	37 (52.9)		0.77 (0.40-1.49)	.432
uu	39 (39.8)	27 (38.6)		1.00**	
Uu+uu	80 (81.6)	64 (91.4)			
Allele frequency					
U	77 (39.3)	49 (35.0)		1.20 (0.77-1.88)	.424
u	119 (60.7)	91 (65.0)		1.00**	
FokI					
FF	48 (49)	39 (55.7)	.614	1.00**	
Ff	38 (38.8)	25 (35.7)		1.23 (0.64-2.38)	.529
ff	12 (12.2)	6 (8.6)		1.62 (0.56-4.72)	.370
Ff+ff	50 (51.0)	31 (44.3)			
Allele frequency					
F	134 (68.4)	103 (73.6)		1.00**	
f	62 (31.6)	37 (26.4)		1.29 (0.80-2.08)	.302

Abbreviations: OR, odds ratio; CI, confidence interval.
* Data are presented as no (%).
** Chi-square P value with continuity correction.

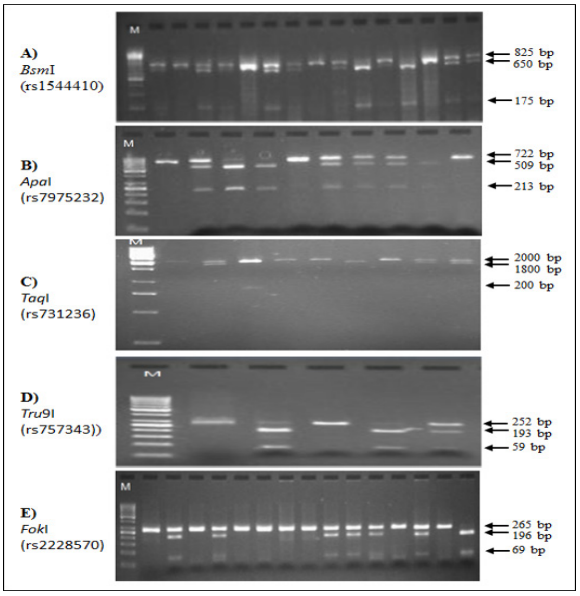

Figure 1. Gel electrophoresis images of the PCR products after digestion with restriction enzymes. A: BsmI polymorphism, B: ApaI polymorphism, C: TaqI polymorphism, D: Tru9I polymorphism, and E: FokI polymorphism. Sizes of the alleles specifying genotypes are given in **Table 1**.

in R software. Possible haplotypes for the BsmI / ApaI / TaqI (rs1544410 / rs7975232 / rs731236) and BsmI/ TaqI (rs1544410 / rs731236) polymorphisms were determined using the HAPSTAT analysis tool, and their relationship to nephrolithiasis was analyzed using the χ^2 test. Odds ratios (ORs) were presented with 95% confidence intervals (CIs), and *P* values less than 0.05 were considered statistically significant. All statistical procedures were performed using the Statistical Package for the Social Science (SPSS Inc, Chicago, Illinois, USA) version 20.0. Statistical power was calculated using QUANTO 1.2.4 software (Website: http://biostats.usc.edu/software).[32] QUANTO is specifically written to calculate either the power or the required sample size for association studies of genes, environmental factors, gene-environment interactions, or gene-gene interactions. In our study, for the less frequent alleles (42.1% for BsmI, 38.6% for ApaI, 35% for TaqI, 35% for Tru9I and 26.4% for FokI) with $P = .05$, the study had a power > 85% for the BsmI, ApaI, TaqI, and Tru9I polymorphisms and > 80% for the FokI polymorphism (OR = 2.0; mode of inheritance: log-additive, population risk: 14.8%).

RESULTS

The characteristics of the subjects are provided in **Table 2**. The mean ages of the patients and normal controls were 47.2 ± 16.3 and 42.6 ± 13.5 years, respectively. The mean body mass index (BMI) (kg/m^2) values of the patients and controls were 24.9 ± 3.7 and 24.1 ± 3.1, respectively.

The genotype and allele distributions of the 5 VDR polymorphisms (BsmI, ApaI, TaqI, Tru9I and FokI) in the nephrolithiasis patients and controls are provided in **Table 3**. None of the genotype frequencies were found to deviate from Hardy-Weinberg equilibrium in either the patients or the controls for the analyzed polymorphisms ($P > .05$).

For the BsmI polymorphism, allele frequency distribution was found to differ significantly between the patients and the controls ($P < .05$). The "B" allele was found to increase the risk of nephrolithiasis by approximately 1.5-fold (OR = 1.55, 95% CI: 1.00-2.40; *P* =

Table 5. BsmI / ApaI / TaqI and BsmI / TaqI haplotypes of the patients and controls.

Haplotypes	Patients (2n = 196) (%)	Controls (2n = 140) (%)	OR (95% CI)	*P* Value
BsmI / ApaI / TaqI				
baT	64 (0.3253)	53 (0.3768)	1.00*	
Bat	81 (0.412)	48 (0.3448)	1.40 (0.84-2.33)	.198
BaT	7 (0.03575)	2 (0.01199)	2.90 (0.58-14.54)	.297
BAT	17 (0.08861)	9 (0.06802)	1.56 (0.64-3.79)	.320
bAT	27 (0.1383)	28 (0.1984)	0.80 (0.42-1.51)	.492
BsmI / TaqI				
bT	91 (0.4639)	81 (0.5755)	1.00*	
Bt	81 (0.4124)	48 (0.3453)	1.50 (0.94-2.39)	.086
BT	24 (0.1237)	11 (0.07914)	1.94 (0.90-4.21)	.089

Abbreviations: OR, odds ratio; CI, confidence interval.
* Referent haplotype.

Table 6. Genotype and allele frequency of the five vitamin D receptor gene polymorphisms in patients with or without family history.*

Variables	Positive (n = 52)	Negative (n = 46)	*P* Value	OR (95 CI)	*P* Value
BsmI					
BB	16 (30.8)	13 (28.3)	.846	1.00**	
Bb	23 (44.2)	23 (50.0)		0.81 (0.32-2.06)	.662
bb	13 (25.0)	10 (21.7)		1.07 (0.35-3.18)	.922
Bb+bb	36 (69.2)	33 (71.7)		0.89 (0.37-2.12)	.786
Allele frequency					
B	55 (52.9)	49 (53.3)		1.00**	
b	49 (47.1)	43 (46.7)		1.01 (0.58-1.78)	.958
ApaI					
AA	23 (44.2)	20 (43.5)	.801	1.00**	
Aa	20 (38.5)	20 (43.5)		0.87 (0.37-2.06)	.751
aa	9 (17.3)	6 (13.0)		1.30 (0.39-4.31)	.662
Aa+aa	29 (55.8)	26 (56.5)		0.97 (0.44-2.16)	.94
Allele frequency					
A	66 (63.5)	60 (65.2)		1.00**	
a	38 (36.5)	32 (34.8)		1.08 (0.60-1.94)	.798
TaqI					
TT	21 (40.4.)	14 (30.4)	.391	1.00**	
Tt	20 (38.5)	24 (52.2)		0.56 (0.27-1.37)	.199
tt	11 (21.1)	8 (17.4)		0.92 (0.29-2.85)	.880
Tt+tt	31 (59.6)	32 (69.6)		0.65 (0.28-1.49)	.305
Allele frequency					
T	62 (59.6)	52 (56.5)		1.00**	
t	42 (40.4)	40 (43.5)		0.88 (0.50-1.55)	.661
Tru9I					
UU	8 (15.4)	10 (21.7)	.583	0.76 (0.25-2.33)	.631
Uu	24 (46.1)	17 (37.0)		1.34 (0.55-3.24)	.514
uu	20 (38.5)	19 (41.3)		1.00**	
Uu+uu	44 (84.6)	36 (78.3)		1.13 (0.50-2.53)	.774
UU+Uu	32 (61.5)	27 (58.7)		0.59 (0.24-1.48)	.261
Allele frequency					
U	40 (38.5)	37 (40.2)		0.93 (0.52-1.65)	.802
u	64 (61.5)	55 (59.8)		1.00**	
FokI					
FF	28 (53.8)	20 (43.5)	.584	1.00**	
Ff	18 (34.6)	20 (43.5)		0.64 (0.27-1.51)	.311
ff	6 (11.6)	6 (13.0)		0.71 (0.20-2.54)	.602
Ff+ff	34 (46.2)	26 (56.5)		0.93 (0.43-2.01)	.862
Allele frequency					
F	74 (71.2)	60 (65.2)		1.00**	
f	30 (28.8)	32 (34.8)		0.76 (0.42-1.39)	.372

Abbreviations: OR, odds ratio; CI, confidence interval.
* Data are presented as no (%).
** Referent genotype/allele.

.048). Likewise, the BB genotype was more frequently observed in the patients than in the controls. However, this difference was not statistically significant (P = .055). Moreover, the frequency of individuals carrying BB+Bb genotypes was more frequent in the patients than in the controls (76.5% vs. 65.7%) (**Table 4**).

The proportional frequencies of the "a" allele of the ApaI polymorphism, the "t" allele of the TaqI polymorphism, the "U" allele of the Tru9I polymorphism, and the "f" allele of the FokI polymorphism were shown to be less than those of their second alleles in the patients and control subjects. However, we did not find any significant differences in the allele and genotype frequencies of the ApaI, TaqI, Tru9I and FokI polymorphisms (**Table 4**).

We also analyzed the haplotypes of the BsmI / ApaI / TaqI polymorphisms and BsmI / TaqI haplotypes. Haplotype analysis revealed that there were 5 common haplotype blocks for the BsmI, ApaI and TaqI polymorphisms, as shown in **Table 5**. Proportionally, the "BAt" and "baT" haplotypes were found to be more common than other haplotypes in the cases and controls, respectively. With regard to the haplotypes of the BsmI and TaqI polymorphisms, the "bT" haplotype frequency was found to be common in both patients and controls. For the haplotype distribution, we did not show statistically significant differences between the cases and the controls for either the BsmI / ApaI / TaqI or the BsmI/ TaqI haplotypes.

Of the 98 patients, 52 of them (17 females and 35 males) had a positive family history of nephrolithiasis, and the remaining patients (15 females and 31 males) were negative. We did not find any differences between family history and VDR gene polymorphisms (**Table 6**).

DISCUSSION

The involvement of the VDR gene locus and its polymorphisms in nephrolithiasis development has been shown in several studies. Initially, a strong linkage was shown between calcium stone formation and the D12S339 marker, which is located near the VDR gene locus on chromosome 12, by Scott and colleagues. using a candidate gene approach.[28] Then, genetic association studies were performed to analyze the relationship between stone formation and VDR gene polymorphisms in different populations,[10-23] and as seen in the literature, contradictory results were obtained for commonly analyzed VDR SNPs. In one of the analyses performed by Bid and colleagues,[10] a significant difference in genotype frequencies for the VDR FokI polymorphism between subjects with calcium oxalate nephrolithiasis and control subjects in the Indian population was shown. In another study, Bid and coworkers analyzed VDR gene FokI and calcitonin receptor (CTR) gene polymorphisms in pediatric stone patients. With regard to the FokI variation, an apparent difference was demonstrated between the pediatric cases and the control group.[11] In a study performed in Taiwan by Liu and colleagues,[12] the FokI variation of the VDR gene was demonstrated to be a meaningful parameter in the clinical appearance of calcium stone formers. The FF genotype of the FokI polymorphism was found to be associated with recurrence of calcium stones and stone development age. However, no significant difference was shown between patients and healthy controls in terms of FokI genotype frequency. Similarly, we found no significant differences in the allele and genotype frequencies for the FokI polymorphism in the study group from Turkish population (**Table 4**).

In the Chinese Han population, Wang and colleagues[13] studied five polymorphisms (FokI, BsmI, DdeI, ApaI and TaqI) of the VDR gene, and they showed an association for only the ApaI polymorphism. The ApaI genotypes were found to differ between groups, and the "a" allele was shown to be related to an increased risk of stone recurrence. In Japanese stone patients, the TaqI "t" allele was shown to correlate with an approximately 5-fold increased risk of stone development and increased urinary calcium levels. However, they were not able to show differences between ApaI polymorphism distribution and severe and recurrent stones.[22] In Korean stone patients, Seo and colleagues did not show any relationship between the AlwI, FokI, ApaI, and TaqI polymorphisms of the VDR gene and urinary stone development.[23]

The BsmI, ApaI, and TaqI genotypes were analyzed in hypercalciuric children from Turkey, and the AA genotype of the ApaI SNP was shown to be related to a 3.5-fold increased risk of idiopathic hypercalciuria.[14] Moreover, in pediatric stone patients and matched controls, Ozkaya and colleagues[15] analyzed VDR ApaI, BsmI and TaqI SNPs and found that ApaI and TaqI variations were related to nephrolithiasis and family history of the disease, respectively. In another study from Turkey, Gunes and colleagues[16] found a relationship between ApaI polymorphisms and family history of stone development. However, in our study, such an association was not found for the ApaI polymorphism for either stone development or family history. However, we found that the bb genotype was present less frequently in stone patients, as shown in **Table 3**.

Gunes and colleagues[16] did not find any significant

differences between urolithiasis patients and controls in the Black Sea Coastal Region of Turkey with respect to genotype frequencies of the BsmI, ApaI and TaqI polymorphisms. With regard to this study, we found a more similar genotype and allele distribution in the healthy control group. Likewise, similar frequencies were also observed in the patients, except for the BsmI polymorphism (B allele 43%; b allele 57%). An approximately 10% difference between the BsmI allele frequencies of the two studies may be attributed to various factors, such as stone type or patient characteristics.

In another study, the bb genotype of the BsmI polymorphism was found to be significantly higher in hypercalciuric stone patients than in normacalciuric stone patients in the Brazilian population.[16] In the Taiwanese population, the BsmI polymorphism was not found to be associated with calcium oxalate stone disease.[20] Heilberg and colleagues showed that the BsmI polymorphism was not related to bone loss in hypercalciuric calcium-stone-forming Brazilian patients.[21]

As seen in the literature, conflicting results have been noted regarding the relationship between VDR gene polymorphisms and nephrolithiasis development. These differences among studies may result from the complex etiology of stone formation, ethnicity, patient characteristics, sample size, environmental effects and other genetic factors.

Recently, a meta-analysis aiming to reveal a possible relationship between VDR gene polymorphisms and nephrolithiasis was performed by Zhou and colleagues.[18] Six studies were included in their analysis, and they showed no association between the VDR BsmI, FokI, TaqI or ApaI polymorphisms and stone formation in either the overall population or Caucasians. However, FokI, TaqI and ApaI polymorphisms were related to the development of nephrolithiasis in the Asian population.[18]

Some alleles of VDR gene polymorphisms also tend to show linkage disequilibrium with each other.[25] Therefore, a haplotype analysis of BsmI, ApaI and TaqI polymorphisms has been performed in some studies. Mossetti and colleagues[19] analyzed the BsmI/TaqI haplotypes and did not show any differences between stone patients and controls with respect to haplotype frequencies. However, they showed that the bT haplotype was associated with an earlier age of onset and elevated stone rate, as well as diminished urinary citrate excretion.

In our study, the BAt, baT and bAT haplotypes of the BsmI / ApaI / TaqI polymorphisms and Bt and bT haplotypes of the BsmI/TaqI polymorphisms were found to have higher frequencies in both patients and controls. However, we did not show statistically significant differences between the groups (**Table 5**).

The limitations of current study include a low number of patients (98), which seems to be insufficient to represent the entire Turkish population. However, when looking at our results, the BsmI polymorphism of the VDR gene is related to nephrolithiasis, and this association may be stronger in a study including a higher number of cases and controls. However, this specific cohort consisted of patients with urinary CaOx stone and for all polymorphisms, the power was > 80%, which was acceptable. Another limitation was the need for more detailed serum and 24 hour urine tests that could be performed for all patients. This would have resulted in a stronger discussion.

CONCLUSIONS

More studies should be performed to reveal the possible association between VDR gene polymorphisms and the risk of nephrolithiasis in a larger cohort. Understanding the genetic tendency toward kidney stone development and recurrence may provide an opportunity for early diagnosis and may also be helpful for the clinical follow-up of urinary stone sufferers.

CONFLICT OF INTEREST

None declared.

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