



## CLINICAL RESEARCH ARTICLE

## Vitamin D receptor gene (FokI, TaqI, BsmI, and ApaI) polymorphisms in children with urinary tract infection

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**BACKGROUND:** Little is known about the genetic background of urinary tract infection (UTI) in children.

**METHODS:** In this study, vitamin D receptor (VDR) gene polymorphisms were compared between 60 children with UTI (case group) and 60 healthy children (control group). DNA extraction, polymerase chain reaction, and the restriction fragment length polymorphism methods were used to perform the genetic analysis.

**RESULTS:** There was a significant difference between the case and control groups for VDR gene, ApaI and BsmI, polymorphisms ( $P < 0.05$ ). The frequency of VDR Bb, bb, Aa, and aa genotypes, and the b and a alleles in the case group was significantly higher than that in the control group ( $P < 0.05$ ). A significant difference was also found between lower UTI and acute pyelonephritis groups for the VDR ApaI and BsmI genotypes ( $P < 0.05$ ). There was no significant difference between children with first UTI and those with more than one UTI for VDR gene polymorphisms ( $P > 0.05$ ).

**CONCLUSION:** This study showed that there is a significant relationship between VDR gene, ApaI and BsmI, polymorphisms and UTI in children. The results indicate that these polymorphisms may play a role in pathogenesis of UTI.

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## INTRODUCTION

Urinary tract infection (UTI) is a common infectious disease in children. Two common forms of UTI can be classified as upper UTI (acute pyelonephritis) and lower UTI (cystitis).<sup>1–3</sup> Although, several factors such as vesicoureteral reflux, urinary system abnormalities, and constipation can increase the risk of UTI, but some patients do not have any known risk factors.<sup>2,3</sup> Such cases raise the question of whether a genetic factor is a risk factor.

It has been reported that gene polymorphisms of some inflammatory molecules, such as polymorphisms of interleukin 6 and interleukin 8, may increase the chances of UTI, and consequently renal scar formation.<sup>4</sup> While considering vitamin D's regulatory immune and antibacterial role,<sup>5</sup> the question of whether the vitamin D receptor (VDR) polymorphisms play a role in the pathogenesis of a UTI arises. Aslan et al. reported that there is a significant relationship between some of the VDR gene polymorphisms and UTI in children.<sup>5</sup>

Vitamin D is a secosteroid hormone that, in addition to calcium and phosphorus homeostasis, plays a mediator's role in immunological and inflammatory processes.<sup>5,6</sup> Several studies have addressed the role of vitamin D in some infectious diseases such as tuberculosis and pneumonia.<sup>7,8</sup> Studies have shown that the biological function of vitamin D is carried out through the VDR.<sup>9,10</sup> It has been reported that there are more than 200 polymorphisms of VDR receptor genes, and the most important of these polymorphisms are FokI, TaqI, BsmI, and ApaI.<sup>6,9–11</sup> Given the role of the aforementioned polymorphisms in some infectious diseases such as tuberculosis and pneumonia,<sup>6,7</sup> the importance of determining the genetic background was presented. Thus, the present study was conducted to determine the relationship between VDR gene (FokI, TaqI, BsmI, and ApaI) polymorphisms and UTI in children.

## METHODS

## Study population

In this study, the VDR gene (FokI, TaqI, BsmI, and ApaI) polymorphisms were tested in 60 children with a final diagnosis of UTI (case group) and were compared with the VDR gene polymorphisms in 60 healthy children (control group). Children were between 1 month and 12 years old. This study was conducted in Qazvin Children's Hospital, which has an affiliation to Qazvin University of Medical Sciences (Qazvin, Iran), in 2016–17. This hospital is the only children's referral hospital in the Qazvin province. Inclusion criteria for the case group were as follows: (1) age: between 1 month and 12 years; (2) the presence of clinical symptoms of UTI, such as fever, anorexia, poor feeding, vomiting, agitation with micturition, abdominal pain, flank pain, dysuria, and frequency; (3) abnormal urinalysis such as pyuria (more than five leukocytes per microscopic field), positive nitrite test, and bacteriuria; and (4) positive urine culture (presence of more than  $10^5$  microorganisms of a single pathogen in 1 ml (milliliter) of urine (CFU/ml) using midstream or clean catch methods, more than  $10^3$  microorganisms in 1 ml (milliliter) of urine (CFU/mL) using catheterization method, and the presence of any number of colonies of an organism in urine culture taken by suprapubic method).<sup>1,3</sup> Children with known risk factors such as vesicoureteral reflux, urinary system abnormalities (such as hydronephrosis, ureteropelvic junction obstruction, and neurogenic bladder), vaginal adhesion, constipation, and any underlying and associated diseases (such as malnutrition and diabetes) were excluded. The control group was selected by the group-matching method from the pool of healthy children who visited the hospital's health clinic for vaccination and growth control or who were hospitalized in the surgical ward for elective surgery. Both groups were similar in

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**Table 1.** Primer sequences, annealing temperature, restriction enzymes, and fragment sizes of the vitamin D receptor gene polymorphisms

VDR polymorphisms	Primers	PCR annealing temperature (°C)	PCR <sup>a</sup> product (bp)	RFLP <sup>b</sup> fragments (bp)	Restriction enzyme
Fok I	F:CACTGACTCTGGCTCTGACCG R:CCTGAAGAAGCCTTTCAGCC	64	195	58,137	Fok I
TaqI	F:AGCAGAGCAGAGTTCCAAGC R:GTGAGGAGGGCTGCTGAGTA	63	701	580,121	TaqI
BsmI	F:ACCTGGCCATTGTCTCTCAC R:CTAACCAGCGGAAGAGGTCA	60	600	422,178	BsmI
ApaI	F:AGCAGAGCAGAGTTCCAAGC R:GTGAGGAGGGCTGCTGAGTA	63	701	303,398	ApaI

<sup>a</sup>PCR (polymerase chain reaction)  
<sup>b</sup>RFLP (restriction fragment length polymorphism)

age and sex. All children lived in Qazvin. This study was approved by the ethics committee in the Qazvin University of Medical Sciences (Ethics no.: IR.Qums.REC.1394.807). All parents were provided information regarding the research method. Children were included in the study after their parents agreed and signed the informed consent form.

**Study design**

Based on the study on children with UTI,<sup>6</sup> our sample size was calculated using  $P_1 = 0.35$  (frequency of FF genotype in control group),  $P_2 = 0.13$  (frequency of FF genotype in case group),  $\alpha$  (error type I) = 0.05,  $\beta$  (error type II) = 0.2, and  $1 - \beta$  (power) = 0.8. Sampling was carried out successively until the sample size was obtained. Dimercaptosuccinic acid (DMSA) renal scan (as the gold standard) was used to distinguish between acute pyelonephritis and acute lower UTI (cystitis). Acute pyelonephritis was confirmed by observing focal or diffuse areas of diminished uptake associated with the preservation of renal cortical outline in the DMSA renal scan. The presence of clinical and laboratory symptoms of UTI associated with normal DMSA renal scan was considered as lower UTI (cystitis).<sup>2,12</sup> The first DMSA renal scan was performed in the first week of admission and the second scan was done 6 months later.

**Genetic analysis**

A volume of 3 ml of peripheral venous whole blood was taken from all children and was put into a tube containing ethylenediaminetetraacetic acid (EDTA), and was kept at  $-20^\circ\text{C}$  until testing time. The DNA was extracted from 200  $\mu\text{l}$  of blood using a High Pure PCR template preparation kit (Roche, Mannheim, Germany, cat. no.: 11796828001). Reaction conditions for the polymerase chain reactions (PCR) using each pair of primers related to four types of VDR receptor gene polymorphisms (including FokI, TaqI, BsmI, and ApaI) were optimized. PCR was performed for 120 samples according to the following protocol: initial denaturation at  $95^\circ\text{C}$  for 5 min, 30 cycles of denaturation at  $95^\circ\text{C}$  for 20 s, annealing for 20 s, and extension at  $72^\circ\text{C}$  for 30 s, and final extension at  $72^\circ\text{C}$  for 5 min. Primer sequence and the annealing temperature suitable for each pair are presented in Table 1. After performing PCR, the specificity of replicated parts was confirmed by electrophoresis on 2% agarose gel. The PCR products for each pair of primers were then cutoff by a specific restriction enzyme (PCR-RFLP method) (Table 1). Following the cutting reaction, the obtained products were electrophoresed. This led to obtaining the length of the cut pieces and determining the related polymorphism.<sup>6,13</sup>

**Statistical analysis**

The Chi-square test and Mann-Whitney *U* test [median (IQR) (interquartile range)] were used, respectively, to compare gender

and age variables between groups. The Chi-square test and odds ratio (OR) with 95% confidence intervals (CI) test were used to compare VDR genotypes among groups. All statistical analyses were performed using SPSS for Windows 16.0 (SPSS Inc., Chicago, IL). *P* values < 0.05 were considered statistically significant.

**RESULTS**

The case group consisted of 10 (16.6%) males and 50 (85.4%) females, and the control group had 12 (20%) males and 48 (80%) females. The minimum, maximum, and median (IQR) age in the case group were 2, 84, and 30 (41.5) months, respectively. These values in the control group were 5, 84, and 30 (38.25) months, respectively. There was no significant difference between the two groups in terms of sex ( $P = 0.63$ ) and age ( $P = 0.91$ ). Amongst the 60 children with UTI, 25 patients had acute pyelonephritis, and 35 patients had lower UTI (cystitis). Amongst the 60 children with UTI, 51 patients experienced UTI for the first time and nine patients had more than one time. A significant difference was found in VDR gene, Bsm1 and Apa1, polymorphisms between the case and the control groups ( $P < 0.05$ ) (Table 2). The frequency of VDR Bb, bb, Aa, and aa genotypes was significantly higher in the case group compared to the control group. [Odds ratio 4.85 (1.69–13.95), 2.73 (1.19–6.27), 2.41 (1.05–5.52), 3.08 (1.14–8.33)] ( $P < 0.05$ ) (Table 2). The risk of UTI was 4.85 times greater in the Bb genotype, 2.73 times greater in the bb genotype, 2.41 times in the Aa genotype, and 3.08 times in the aa genotypes ( $P < 0.05$ ) (Table 2). The frequency of b and a alleles in the case group was significantly higher than that in the control group, and the frequency of B and A alleles in the control group was significantly higher than that in the case group ( $P < 0.05$ ) (Table 2). There was a significant difference between acute pyelonephritis and lower UTI groups in relation to VDR gene, Bsm1 and Apa1, polymorphisms ( $P < 0.05$ ) (Table 3). The frequency of VDR bb and aa genotypes in the lower UTI group was significantly lower than that in the acute pyelonephritis group [odds ratio 0.15 (0.03–0.68), 0.16 (0.03–0.71)] ( $P < 0.05$ ) (Table 3). The frequency of t, b, and a alleles in the acute pyelonephritis group was significantly higher than that in the lower UTI group (Table 3). The frequency of t, B, and A alleles in the lower UTI group was also significantly higher than in the acute pyelonephritis group ( $P < 0.05$ ) (Table 3). No significant difference was found between children with first UTI and those children with more than one UTI in relation to VDR gene polymorphisms and allelic frequency ( $P > 0.05$ ). The frequency of VDR Bb genotype was significantly higher in lower UTI group than in the control group ( $P < 0.05$ ) (Table 4).

Furthermore, a comparison of the acute pyelonephritis group with the control group revealed that there was a higher frequency of VDR Bb, bb, Aa, and aa genotypes in the acute pyelonephritis

**Table 2.** Comparison of vitamin D receptor gene polymorphisms and allelic frequencies between the case and the control groups

VDR genotype/ allelic frequency	Control n (%) total = 60	Case n (%) total = 60	P	Odds ratio (95% CI)	P
<b>FokI</b>					
FF	36 (60)	38 (63.3)	0.21	1	
Ff	17 (28.3)	20 (33.3)		1.11 (0, 5–2.45)	0.78
ff	7 (11.7)	2 (3.3)		0.27 (0.05–1.39)	0.09
<b>TaqI</b>					
TT	29 (48.3)	27 (45)	0.92	1	
Tt	20 (33.3)	22 (36.7)		1.18 (0.53–2.63)	0.68
tt	11 (18.3)	11 (18.3)		1.07 (0.4–2.88)	0.88
<b>BsmI</b>					
BB	34 (56.7)	17 (28.3)	0.004	1	
Bb	7 (11.7)	17 (28.3)		4.85 (1.69–13.95)	0.002
bb	19 (31.7)	26 (43.3)		2.73 (1.19–6.27)	0.01
<b>ApaI</b>					
AA	33 (55)	19 (31.7)	0.03	1	
Aa	18 (30)	25 (41.7)		2.41 (1.05–5.52)	0.03
aa	9 (15)	16 (26.7)		3.08 (1.14–8.33)	0.02
<b>FokI</b>					
F	89 (74.2)	96 (80)			0.28
f	31 (25.8)	24 (20)			
<b>TaqI</b>					
T	78 (65)	76 (63.3)			0.78
t	42 (35)	44 (36.7)			
<b>BsmI</b>					
B	75 (62.5)	51 (42.5)			0.001
b	45 (37.5)	69 (57.5)			
<b>ApaI</b>					
A	84 (70)	63 (52.5)			0.005
a	36 (30)	57 (42.5)			

Pearson chi-square test

**Table 3.** Comparison of vitamin D receptor gene polymorphisms and allelic frequencies between the acute pyelonephritis and the lower urinary tract infection groups

VDR genotype/ allelic frequency	Acute pyelonephritis n (%) total = 25	Lower UTI (cystitis) n (%) total = 35	P	Odds ratio (95% CI)	P
<b>FokI</b>					
FF	14 (56)	24 (68.6)	0.19	1	
Ff	11 (44)	9 (25.7)		0.47 (0.15–1.43)	0.18
ff	0 (0)	2 (5.7)		—	0.28
<b>TaqI</b>					
TT	15 (60)	12 (34.3)	0.13	1	
Tt	7 (28)	15 (42.9)		2.67 (0.82–8.67)	0.09
tt	3 (12)	8 (22.9)		3.33 (0.72–15.37)	0.11
<b>BsmI</b>					
BB	3 (12)	14 (40)	0.03	1	
Bb	7 (28)	10 (28.6)		0.3 (0.06–1.48)	0.13
bb	15 (60)	11 (31.4)		0.15 (0.03–0.68)	0.009
<b>ApaI</b>					
AA	4 (16)	15 (42.9)	0.04	1	
Aa	11 (44)	14 (40)		0.33 (0.08–1.31)	0.11
aa	10 (40)	6 (17.1)		0.16 (0.03–0.71)	0.01
<b>FokI</b>					
F	39 (78)	57 (81.4)			0.64
f	11 (22)	13 (18.6)			
<b>TaqI</b>					
T	37 (74)	39 (55.7)			0.04
t	13 (26)	31 (44.3)			
<b>BsmI</b>					
B	13 (26)	38 (54.3)			0.002
b	37 (74)	32 (45.7)			
<b>ApaI</b>					
A	19 (38)	44 (62.9)			0.007
a	31 (62)	26 (37.1)			

Pearson chi-square test

group [11.3 (2.3–54.9), 8.94 (2.29–34.8), 5.04 (1.4–18.1), 9.16 (2.32–36.2)] ( $P < 0.05$ ) (Table 5). Among 25 children with acute pyelonephritis who underwent DMSA renal scan and a complete antibiotic treatment for 2 weeks, only eight children accepted to re-conduct the DMSA renal scan in the following 6 months. Among these eight children, seven had normal DMSA renal scans and in one child, the renal scar formation was noted. Renal scar formation was found in a 2.5-year boy who had been suffering from UTI for the first time. The cause of UTI in this patient was *Escherichia coli*. The characteristics of VDR gene polymorphisms in this patient were Ff, bb, aa, and TT genotypes.

**DISCUSSION**

A greater understanding of the pathogenesis of UTI is a crucial step in establishing therapeutic strategies and preventive measures for this disease. There are few studies concerning the role of genetic factors in UTI. The study of Aslan et al. on 92 children with UTI (case group) and 105 healthy children (control

group) with mean ages of  $7.3 \pm 3.6$  and  $14.1 \pm 2.2$  years showed that there is a significant difference between case and control groups in relationship to VDR FokI polymorphism frequency. Arslan et al. reported that the VDR FokI polymorphism to be a risk factor for UTI and the VDR ApaI polymorphism to be a protective factor. Their study also revealed that there is no significant difference between the acute pyelonephritis group and the lower UTI group regarding VDR gene polymorphisms. The distribution of polymorphisms in three groups of pyelonephritis with scar, pyelonephritis without scar, and the control group was the same. These researchers showed that VDR Ff, ff, and Bb genotypes and VDR Ff and ff genotypes are risk factors for lower UTI and pyelonephritis with scar groups, respectively. They also reported that VDR Aa and aa genotypes are protective factors for each of the three groups of lower UTI, pyelonephritis with scars, and pyelonephritis without scar.<sup>6</sup>

**Table 4.** Comparison of vitamin D receptor gene polymorphisms and allelic frequencies between the lower urinary tract infection and the control groups

VDR genotype/ allelic frequency	Control n (%) total = 60	Lower UTI (cystitis) n (%) total = 35	P	Odds ratio (95% CI)	P
<b>FokI</b>					
FF	36 (60)	24 (68.6)	0.56	1	
Ff	17 (28.3)	9 (25.7)		0.79 (0.3–2.07)	0.63
ff	7 (11.7)	2 (5.7)		0.42 (0.08–2.24)	0.3
<b>TaqI</b>					
TT	29 (48.3)	12 (34.3)	0.41	1	
Tt	20 (33.3)	15 (42.9)		1.81 (0.7–4.6)	0.21
tt	11 (18.3)	8 (22.9)		1.75 (0.56–5.45)	0.32
<b>BsmI</b>					
BB	34 (56.7)	14 (40)	0.09	1	
Bb	7 (11.7)	10 (28.6)		3.46 (1.1–10.9)	0.02
bb	19 (31.7)	11 (31.4)		1.4 (0.53–3.7)	0.48
<b>ApaI</b>					
AA	33 (55)	15 (42.9)	0.5	1	
Aa	18 (30)	14 (40)		1.71 (0.67–4.32)	0.25
aa	9 (15)	6 (17.1)		1.46 (0.44–4.86)	0.53
<b>FokI</b>					
F	89 (74.2)	57 (81.4)			0.25
f	31 (25.8)	13 (18.6)			
<b>TaqI</b>					
T	78 (65)	39 (55.7)			0.2
t	42 (35)	31 (44.3)			
<b>BsmI</b>					
B	75 (62.5)	38 (54.2)			0.26
b	45 (37.5)	32 (45.8)			
<b>ApaI</b>					
A	84 (70)	44 (62.8)			0.31
a	36 (30)	26 (37.2)			

Pearson chi-square test

Contrary to the study of Aslan et al., our study showed that there is a significant difference between the case and the control groups for VDR gene, ApaI and BsmI, polymorphisms. It seems that these polymorphisms may be risk factors for UTI. In our study, the probability of UTI occurrence with genotype Bb was 1.8 times higher than bb genotype. With aa genotype, the probability was 1.2 times higher than Aa genotype. The present study also showed that b and a alleles are associated with UTI, and B and A alleles have protective roles. Contrary to the study of Aslan et al, our study showed that there is a significant difference between the lower UTI and the acute pyelonephritis groups for VDR gene, ApaI and BsmI, polymorphisms. The risk of lower UTI with these genotypes was 0.16 and 0.15 times lower. It seems that VDR aa and bb genotypes have protective roles for lower UTI. Also, our study showed that VDR gene polymorphisms had no role as risk or protective factors for recurrent UTI. Since only one case of renal scar formation was found in the repeated DMSA renal scan

**Table 5.** Comparison of vitamin D receptor gene polymorphisms and allelic frequencies between acute pyelonephritis and control groups

VDR genotype/ allelic frequency	Control n (%) total = 60	Acute pyelonephritis n (%) total = 25	P	Odds ratio (95% CI)	P
<b>FokI</b>					
FF	36 (60)	14 (56)	0.11	1	
Ff	17 (28.3)	11 (44)		1.66 (0.62–4.42)	0.35
ff	7 (11.7)	0 (0)		—	0.17
<b>TaqI</b>					
TT	29 (48.3)	15 (60)	0.59	1	
Tt	20 (33.3)	7 (28)		0.67 (0.23–1.95)	0.47
tt	11 (18.3)	3 (12)		0.52 (0.12–2.1)	0.37
<b>BsmI</b>					
BB	34 (56.7)	3 (12)	0.001	1	
Bb	7 (11.7)	7 (28)		11.3 (2.3–54.9)	0.001
bb	19 (31.7)	15 (60)		8.94 (2.29–34.8)	0.0004
<b>ApaI</b>					
AA	33 (55)	4 (16)	0.002	1	
Aa	18 (30)	11 (44)		5.04 (1.4–18.1)	0.009
aa	9 (15)	10 (40)		9.16 (2.32–36.2)	0.001
<b>FokI</b>					
F	89 (74.2)	39 (78)			0.59
f	31 (25.8)	11 (22)			
<b>TaqI</b>					
T	78 (65)	37 (74)			0.25
t	42 (35)	13 (26)			
<b>BsmI</b>					
B	75 (62.5)	13 (26)			0.0001
b	45 (37.5)	37 (74)			
<b>ApaI</b>					
A	84 (70)	19 (38)			0.0001
a	36 (30)	31 (62)			

Pearson chi-square test

operated on patients with acute pyelonephritis in the next 6 months, it was not possible for this study to investigate the role of VDR gene polymorphisms in the development of renal scar. Other studies have shown the relationship between VDR gene polymorphisms and other infectious diseases in children.<sup>14–20</sup> Han et al. have shown that in 166 patients with pertussis, the frequency of VDR major allele and its homozygous genotypes was significantly higher in patients with symptomatic pertussis than in the control group. The relationship between VDR major allele and duration of pertussis symptoms was statistically significant. The study concluded that VDR gene polymorphisms had an effect on the clinical outcome of *B. pertussis* infection.<sup>14</sup> A study by Roth et al. on 56 children with lower respiratory tract infection and 64 healthy children showed that the chance of incidence of lower respiratory tract infections with the FokI ff genotype is seven times higher than that in the FokI FF genotype. The study suggested that there is a weak association between the

two groups for VDR TaqI polymorphism. The researchers concluded that VDR genotypes are risk factors for lower respiratory tract infections.<sup>15</sup> The study by Areeshi et al. showed that VDR ApaI polymorphism has a protective role in the prevention of pulmonary tuberculosis in the African population, but not the Asian one.<sup>16</sup> Moreover, the risk factor role for T allele of the VDR TaqI polymorphism in the development of tuberculosis was highlighted by the studies of Cao et al.<sup>17</sup> Another study by Banoei et al. indicated the risk factor role of tt and bb genotypes of VDR TaqI and BsmI polymorphisms in the incidence of pulmonary tuberculosis in Iranian population.<sup>18</sup> Motsinger-Reif et al. and Zhang et al. showed the relationship between VDR FokI polymorphism, and extra-PTB and spinal TB in the American and Chinese populations, respectively.<sup>19,20</sup> The different results obtained by these studies can be attributed to some factors such as ethnicity and diet. Based on some reports, these factors play a major role in the distribution of VDR gene polymorphisms in human societies.<sup>21–23</sup> Haddad reported that the highest frequency of VDR gene, Apa-I, polymorphism (genotype AA) is present in the black communities of Pennsylvania, Syria, Jordan, and Turkey, and the lowest frequency is in the Japanese, Chinese, and Thai populations, respectively.<sup>21,23</sup> In some studies, a significant relationship was found between VDR gene polymorphisms and calcium metabolism disorders.<sup>22</sup>

Vitamin D is a vital dietary element for human health and has two major forms; vitamin D2 and vitamin D3. Sunlight radiation on the skin induces the conversion of 7-dehydrocholesterol (7-DHC) to vitamin D3. Hydroxylation of vitamin D3 in the liver by the cytochrome P450 enzyme CYP2R1 and in the kidney by the enzyme CYP27B1 produces 25-hydroxyvitamin D3 and 1, 25-dihydroxyvitamin D3.<sup>24,25</sup> The 1, 25-dihydroxyvitamin D3 hormone is an active form of vitamin D. In addition to controlling calcium-phosphorus homeostasis and metabolism, vitamin D has various extra-bone activities such as modulating the activity of defense and immune cells, including lymphocytes, monocytes, macrophages, and epithelial cells. Vitamin D can also have an effect on the development of various diseases, including infectious diseases, by increasing phagocytosis via macrophage activation and consequently affecting the immune system.<sup>13,25–28</sup>

Several studies have pointed to the role of vitamin D in innate and adaptive immunity.<sup>25–28</sup> It is believed that target cells such as monocytes and macrophages not only express VDR, but also the VitD-activating enzyme or CYP27B1. These cells consume circulating 25-dihydroxyvitamin D3 for intracellular activity that induces the antimicrobial reply to invasive microorganisms (such as cathelicidin and defensin β2). These cells also recognize pathogen-associated molecular patterns via their toll-like receptors. These receptors upregulate the expression of genes that code for the VDR and CYP27B1.<sup>25–28</sup> The role of vitamin D on innate immunity of the urinary tract system has been evaluated by Hertting et al.<sup>29</sup> They showed that administration of oral 25-hydroxyvitamin D3 to healthy postmenopausal women increases the ability of the bladder tissue to fight with *E. coli* by increased production of cathelicidin. 25-hydroxyvitamin D3 is locally changed to 1, 25 hydroxyvitamin D3 in bladder epithelial cells and then binds to VDR, which leads to the upregulation of CAMP and synthesis of cathelicidin. Cathelicidin has a direct antibacterial effect on uropathogenic *E. coli*.<sup>29</sup> Furthermore, it has been shown that 1,25-dihydroxyvitamin D3 has an effect on both T- and B-cell immune responses via multiple mechanisms such as modulation of T-cell antigen receptors and inhibition of T- and B-cell proliferation.<sup>25</sup>

1,25-dihydroxyvitamin D3 hormone exerts its effect through VDR.<sup>10,25,30</sup> The calcitriol receptor, more commonly known as the VDR and also known as NR1H1 (nuclear receptor subfamily 1, group I, member 1), is a member of the nuclear receptor family of transcription factors. This receptor is located on the chromosome 12cen-ql2 and consists of 14 exons and spans ~75 kilobases of

genomic DNA.<sup>11,31</sup> 1,25-dihydroxyvitamin D3, the active form of vitamin D, binds to VDR, which then forms a heterodimer with the retinoid-X receptor. This then binds to hormone-response elements on DNA, resulting in expression or transcription of specific gene products. VDR not only regulates transcriptional responses, but is also involved in micro RNA-directed post-transcriptional mechanisms. In humans, the VDR is encoded by the VDR gene.<sup>32,33</sup> VDR gene is considered as a candidate locus for incidence of various diseases due to the effect of allele diversity on receptor activity.<sup>6,14–20,30,33,34</sup> Some studies have reported a link between vitamin D status, response to vitamin D supplementation, and VDR gene polymorphisms.<sup>35–39</sup> Studies by Martineau et al., Søborg et al., and Lewis et al. on pulmonary tuberculosis patients have shown that administration of high doses of VitD have no effect on sputum conversion time when assessed in relation to FokI genotype,<sup>35</sup> while other VDR SNPs appeared to influence the response to VitD supplementation.<sup>36,37</sup> The Karpinski study on low-energy bone fractures has demonstrated that ApaI polymorphism recessive “aa” and TaqI polymorphism dominant “TT” genotypes are associated with higher levels of vitamin D in serum.<sup>38</sup> A study on Egyptian obese women with vitamin D deficiency has verified that VDR polymorphisms plays an important role in immune and inflammation status.<sup>39</sup>

Although, this study highlighted the possible risk factor of VDR gene, ApaI, BsmI, polymorphisms for UTI in children, more studies are needed in this area. In addition, we recommend simultaneous measurement of serum vitamin D level and polymorphisms in patients affected by UTI in future studies.

This study's limitations included small sample size, lack of measurement of serum vitamin D levels, and the lack of investigation of the association between VDR gene polymorphisms and renal scar formation resulting from acute pyelonephritis.

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## ADDITIONAL INFORMATION

**Competing interests:** The authors declare no competing interests.

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