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CLINICAL STUDY

## The role of vitamin D receptor gene polymorphisms in Turkish infants with urolithiasis

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### ABSTRACT

Polymorphisms in the vitamin D receptor (VDR) gene have recently been reported to be associated with urinary calculi in pediatric and adult cases, but no studies have looked at the youngest period of life. The purpose of this study was to investigate the role of VDR gene polymorphisms in infantile urolithiasis in a Turkish population. We compared a study group of 104 infants (55 girls and 49 boys, mean age  $6.94 \pm 3.81$  months) with a control group of 96 infants (51 girls and 45 boys, mean age  $7.51 \pm 3.23$ ) to evaluate their demographics and metabolic risk factors. PCR-based restriction analysis of the polymorphisms on the VDR gene (BsmI and TaqI) showed statistically significant differences between study and control groups ( $p = 0.001$  and  $0.043$ , respectively). In addition, the prevalence of the BsmI genotype was significantly different between the hypercalciuric and normocalciuric stone formers ( $p = 0.007$ ). Allelic frequencies were similar between the urolithiasis and control groups ( $p > 0.05$ ). The B allele of BsmI and the A allele of Apal were more prevalent in the hypercalciuric stone formers than in the normocalciuric stone formers ( $p = 0.018$  vs.  $0.036$ , respectively). These results suggest that the BsmI and TaqI VDR genotypes could be candidate genes leading to infantile urolithiasis.

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## Introduction

The prevalence of urolithiasis is rising, both in countries in which stone disease is endemic, such as Turkey, and also in non-endemic ones,<sup>1–5</sup> either because of increasing awareness or because of the greater use of radiologic diagnostic techniques. Factors leading to urolithiasis include metabolic abnormalities, genetics, nutrition, environment, and specific medicines.<sup>6,7</sup> Hypercalciuria is the most common metabolic abnormality.<sup>2,8</sup> Infantile urolithiasis represents 9–23% of all pediatric cases; approximately, 60% of infants have family history of stone disease.<sup>1,2,8</sup>

While knowledge is limited on how genetic factors mediate susceptibility to urolithiasis, one important factor is the function of the vitamin D receptor (VDR). Vitamin D plays a critical role in intestinal calcium absorption and renal calcium reabsorption,<sup>9</sup> and the VDR, a member of the steroid receptor family, mediates

effects of the active metabolite 1,25-dihydroxyvitamin D<sub>3</sub>.<sup>10</sup> More than 60 polymorphisms have been reported in the VDR gene,<sup>11</sup> primarily related to such diseases as cancer, renal diseases, and diabetes.<sup>12</sup> An increase in the number of VDR has been observed in hypercalciuric rats, and a susceptibility locus has been defined in hypercalciuric patients.<sup>13,14</sup> These findings suggest that allelic variation of the VDR gene could be responsible for the genetic susceptibility associated with urolithiasis. Other studies have evaluated the association of the VDR gene with urolithiasis, hypercalciuria, and hypocitraturia,<sup>9,12,15</sup> but not for infantile urolithiasis.

The aim of this study is to evaluate urolithiasis in the first year of life, its risk factors, demographic factors, and relationship with VDR gene polymorphisms. Since genotype and allele frequencies are known to vary in different ethnic populations, we focused our investigation on BsmI, Apal, and TaqI in urolithiasis of infants in Turkey.

## Materials and methods

The study group comprised 104 infants (55 girls and 49 boys, mean age  $6.94 \pm 3.81$  mo) who, in our pediatric nephrology clinic, had been diagnosed by ultrasound (US) as having urolithiasis. Microlithiasis was defined as calculi  $< 3$  mm in US examination. Small and newly developed stones, however, may not produce posterior shadowing, especially if less than 3 mm. Infants were fed before US, and those with fever or an acute illness, such as diarrhea were excluded. Their mean birth weight was  $2971.5 \pm 682$  g. Ten of the 104 were born prematurely, but they were not excluded from the study group because prematurity is a known risk factor for stone formation, shown in a recent study to constitute 12% of cases.<sup>2</sup> The control group included 96 age- and gender-matched healthy infants (51 girls and 45 boys, mean age  $7.51 \pm 3.23$  mo) proven to be stone-free by USG. Patients with abnormal renal function and renal tubular acidosis were recruited from the control and study groups.

Each subject's medical history included age, gender, weight, height, birth history, length of breastfeeding in months, time of the start of supplementary feeding, average daily dosage of vitamin D supplementation, previous diagnosis of urinary tract infection, and symptoms, such as restlessness, crying, vomiting, and hematuria. Serum samples were obtained for routine tests. For metabolic investigation, urine was analyzed for calcium, uric acid, oxalate, and citrate; blood was analyzed for vitamin D gene polymorphism, venous blood gas, calcium, phosphorus, 25 hydroxy vitamin D level, alkaline phosphatase, potassium, uric acid, creatinine, and sodium. Measurements for citrate and oxalate were made in the hospital's clinical laboratory using standard enzymatic and photometric methods (autoanalyzer, Cobas 6000, Roche, Ben S.r.l. Via Toselli<sup>®</sup>, 4 20, 127 Milano, Italy and Trinity Biotech Oxalate<sup>®</sup>, Bray, Ireland, respectively).

A ratio of spot values to creatinine was used to rule out the influence of urinary dilution or concentration. Normal spot urine calcium/creatinine ratios were defined as  $< 0.8$  mg/mg creatinine for infants 0–6 mo and  $< 0.6$  mg/mg for infants 7–12 mo. Normal spot urine oxalate/creatinine levels were accepted as  $< 0.28$ – $0.26$  mg/mg creatinine for infants  $< 6$  mo,  $< 0.11$ – $0.14$  mg/mg creatinine for infants 7–24 mo, and spot urine uric acid  $< 2.2$  for infants  $< 1$  year. For hypocitraturia, a spot urine citrate/creatinine ratio of 0.42 mg/mg creatinine was used.<sup>1</sup>

Blood samples added to test tubes containing EDTA were used to isolate the genomic DNA from peripheral blood leukocytes by means of a DNA isolation kit (Invitrogen, Carlsbad, CA). All purified DNA samples were stored at  $+4^{\circ}\text{C}$  until PCR application. DNA could be

obtained from 78 infants in the study group and 60 infants in the control group.

Vitamin D receptor gene polymorphisms (the BsmI and Apal sites in intron 8 and the TaqI site in exon 9) were analyzed using the previously described PCR-restriction fragment length polymorphism (PCR-RFLP) assay, but with minor modifications.<sup>16</sup> We used 25  $\mu\text{L}$  total PCR mixtures containing 10.0 pmol of each primer, specifically, 1.0 mM dNTP (deoxynucleotide triphosphates), 25 mM  $\text{MgCl}_2$ , 2.5U Taq DNA polymerase in the supplied reaction buffer [Taq Buffer with  $(\text{NH}_4)_2\text{SO}_4$ ], and 100–200 ng DNA. PCR was performed with the forward and reverse primers (Table 1), with the initial denaturation at  $94^{\circ}\text{C}$  for 5 min, following 33 cycles of  $94^{\circ}\text{C}$  for 30 s,  $65^{\circ}\text{C}$  for 30 s,  $72^{\circ}\text{C}$  for 30 s, and  $72^{\circ}\text{C}$  for 5 min. PCR products become visible on 2% agarose gel with ethidium bromide staining, digested by restriction enzymes and then genotyped. Three genotypes for each polymorphism were determined by their distinct banding patterns (Figure 1). The BsmI band patterns (#FD0964, Thermo Scientific, Waltham, MA) were 825 bp for the BB (AA) genotype; 825, 650, and 175 bp for the Bb (AG) genotype; and 650, 175 bp for the bb (GG) genotype. The Apal band patterns (#FD1,414, Thermo Scientific) were 740 bp for the AA (TT) genotype; 740, 520, and 220 bp for the Aa (TG) genotype; and 520, 220 bp for the aa (GG) genotype. The TaqI band patterns (#FD0,674, Thermo Scientific) were 495, 245 bp for the TT (TT) genotype; 495, 290, 245, and 205 bp for the Tt (TC) genotype; and 290, 245, 205 bp for the tt (CC) genotype.<sup>16</sup>

The genotype analysis was performed using Pearson's chi-square test. Statistical analyses were performed with SPSS version 17.0 (Chicago, IL). Data are presented as the mean  $\pm$  standard deviation. Differences in variable means between the stone-forming group and the control group were compared by Student's *t*-test. A *p*-value  $< 0.05$  was considered statistically significant.

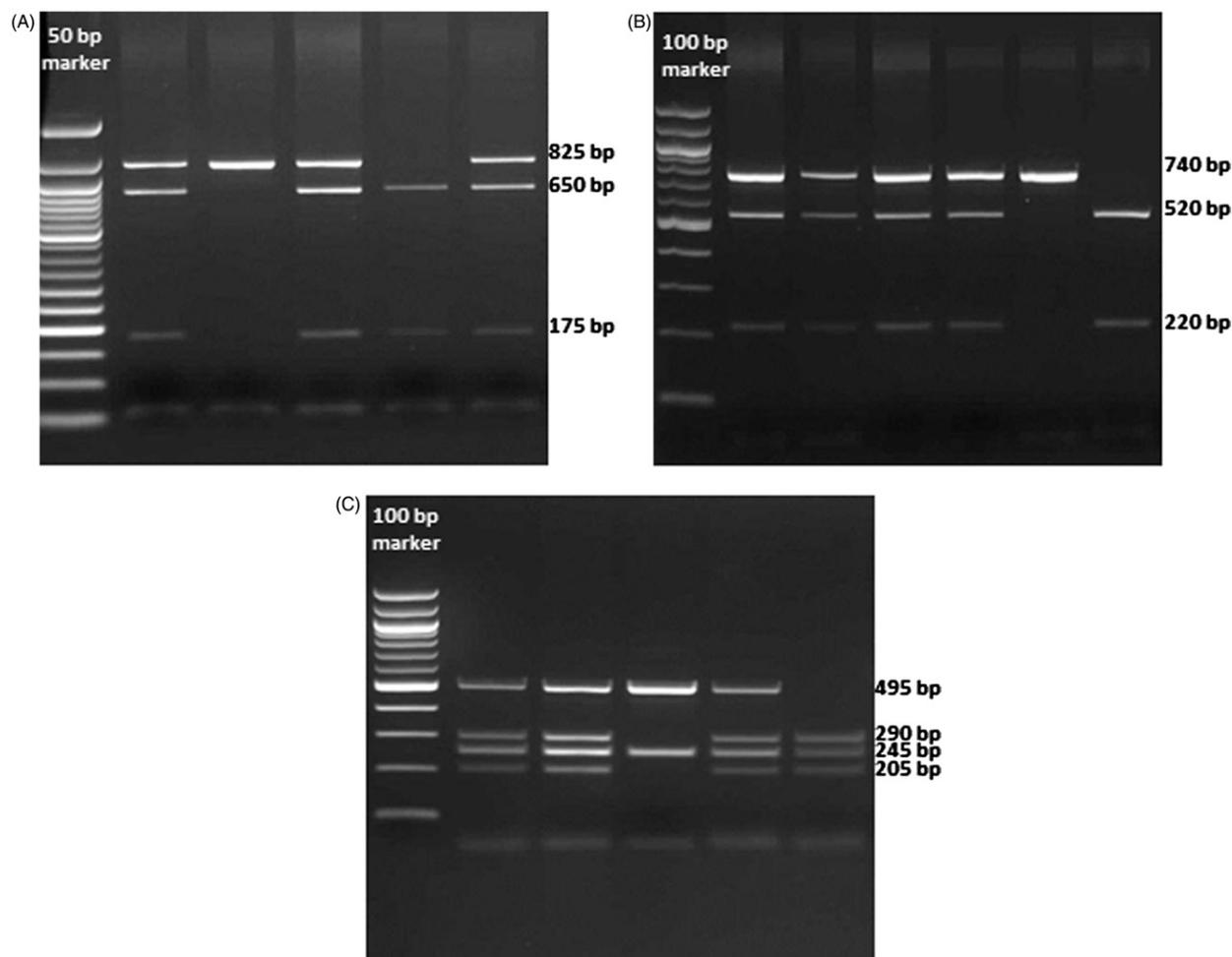
The study was approved by our local ethics committee. Written informed consent was obtained from one or both parents of each infant.

## Results

Gender distribution and the comparisons of mean ages, weight, height, and birth weight revealed no statistically

**Table 1.** Primer sequences in VDR gene polymorphisms.

Polymorphism	Primer sequences
BsmI	Forward primer: CAACCAAGACTACAAGTACCCGCTCAGTGA Reverse primer: AACCAGCGGGAAGAGGTCAAGGG
Apal	Forward primer: CAGAGCATGGACAGGGAGCAAG Reverse primer: GCAACTCCTCATGGCTGAGGTCTCA
TaqI	Forward primer: CAGAGCATGGACAGGGAGCAAG Reverse primer: GCAACTCCTCATGGCTGAGGTCTCA



**Figure 1.** Genotyping for the BsmI, Apal, and TaqI polymorphisms in the VDR gene. (A) Representative gel showing the genotype for BsmI. The first lane of gel contains a 50 bp DNA ladder. (B) Representative gel showing the genotype for Apal. The first lane of gel contains a 100 bp DNA ladder. (C) Representative gel showing the genotype for TaqI. The first lane of gel contains a 100 bp DNA ladder.

significant differences between the two groups ( $p > 0.05$ ) (Table 2). Family history of stone disease was detected in 55% of the infants with urolithiasis. In the study group, 43% of infants were exclusively breastfed, 1% was on formula, and 55% were mixed fed; control group percentages were 58, 13, and 27%, respectively. The study group's daily vitamin D supplementation was slightly higher than that of the controls ( $499.48 \pm 126.58$  vs.  $459.45 \pm 124.96$ , units/day;  $p = 0.042$ ).

The clinical characteristics of infants with urolithiasis are shown in Table 3. Mean stone diameter was 3.36 (1–14 mm); 30 infants had stones with a diameter  $> 3$  mm. Locations of the stones were 15% on right kidneys, 55% on left kidneys, and 30% on both kidneys.

Infants with urolithiasis most commonly presented with restlessness (53.9%). Vomiting was present in 23 patients (22.5%), low appetite in 25 (24.5%), and hematuria in 10 (9.8%). Hydronephrosis were detected in 3, pelvicaliectasia in 2, and renal ectopia in 1 infant. UTI was present in 44 infants.

The study and control groups did not statistically differ in levels of serum calcium (ca), phosphate, 25 hydroxyvitamin D (25OHD), alkaline phosphatase (ALP), uric acid, creatinine, and serum electrolytes (Table 4). The urinary calcium/creatinine ratio was significantly higher in stone formers compared with the stone-free control group ( $p = 0.021$ ), but their urinary excretions of oxalate, citrate, and uric acid did not statistically differ (Table 3). Among the stone-formers, hypercalciuria was detected in 21, hypocitraturia in 10, hyperuricosuria in 7, and hyperoxaluria in 2 patients. Three infants had more than one metabolic abnormality: one had both hypercalciuria and hyperuricosuria, one had both hyperuricosuria and hypocitraturia, and one had hypercalciuria along with hypocitraturia. Also in the study group, 3 patients had elevated serum uric acid levels, 8 had hypercalcemia ( $\text{Ca} > 11$  mg/dL), and one had an elevated vitamin D level. Serum phosphorus levels were normal in all the study group infants.

We further analyzed the study group and divided into two groups according to calculi dimensions. A comparison of the demographic and clinical features of infants with microlithiasis ( $\leq 3$  mm) and stones ( $> 3$  mm) disclosed no statistically significant differences for gender, associated symptoms, or metabolic features.

### Genotype and allele frequencies for *BsmI*

As evident in Table 5, children with urolithiasis had a different VDR-*BsmI* genotype distribution from the

**Table 2.** Demographic and anthropometric features of the study and control groups.

	Study group (n=104)	Control group (n=96)	p
	Mean $\pm$ SD	Mean $\pm$ SD	
Age (months)	6.94 $\pm$ 3.8	7.51 $\pm$ 3.2	0.260
Gender (Female/male)	55/49	51/45	0.923
Weight (kg)	7.5 $\pm$ 1.7	8 $\pm$ 1.8	0.153
Height (cm)	65.3 $\pm$ 6.9	67.9 $\pm$ 9.2	0.084
Birth weight (g)	2971.5 $\pm$ 682	3162.5 $\pm$ 529.2	0.055

**Table 3.** The clinical characteristics of the infants with urolithiasis.

	n (%)
Stones $\leq 3$ mm	74 (71)
Stones $> 3$ mm	30 (29)
Family history of urolithiasis	57 (55)
Presenting symptoms	
UTI	44 (42)
Restlessness	56 (53.9)
Vomiting	23 (22.5)
Low appetite	25 (24.5)
Hematuria	10 (9.8)
Urinary metabolic examination	
Hypercalciuria	21 (20)
Hyperoxaluria	2 (1.9)
Hypocitraturia	10 (9.6)
Hyperuricosuria	7 (6.7)

**Table 4.** Serum and urine metabolic markers of the stone-forming children and controls.

	Study group (n=104)	Control group (n=96)	p
Serum			
Calcium (mg/dL)	10.3 $\pm$ 0.6	10.4 $\pm$ 0.4	0.080
Phosphate (mg/dL)	5.5 $\pm$ 0.6	5.5 $\pm$ 0.5	0.564
ALP (U/L)	231.1 $\pm$ 76.3	215.1 $\pm$ 72.1	0.168
Uric acid (mg/dL)	3.3 $\pm$ 1.1	3.1 $\pm$ 0.7	0.220
25OHD (ng/mL)	36.8 $\pm$ 15.6	33.6 $\pm$ 14.3	0.161
Creatinine (mg/dL)	0.2 $\pm$ 0.1	0.2 $\pm$ 0.1	0.490
Sodium	137.3 $\pm$ 10.7	137.7 $\pm$ 2.1	0.698
Urine			
Calcium/creatinine	0.5 $\pm$ 0.3	0.3 $\pm$ 0.2	<b>0.021</b>
Oxalate/creatinine	83.5 $\pm$ 50	92.5 $\pm$ 79.3	0.424
Uric acid/creatinine	1.6 $\pm$ 1.1	1.5 $\pm$ 0.5	0.426
Citrate/creatinine	1136.7 $\pm$ 941.8	1464.2 $\pm$ 1231.3	0.100

ALP (U/L): alkaline phosphatase, 25OHD: 25 hydroxyvitamin D. Statistical analysis: Student's *t*-test. *p* value  $< 0.05$  was considered as significant.

control group ( $p = 0.001$ ); therefore, we further analyzed subdivisions of the two groups and found that the *bb* genotype was greater than the *BB+Bb* genotype ( $p < 0.001$ ). Allelic frequencies were not significantly different between infants with urolithiasis and the controls ( $p = 0.065$ ). The hypercalciuric infants with urolithiasis have a different distribution of the *BsmI* genotype than the infants with normocalciuric urolithiasis (Table 6) ( $p = 0.007$ ). Both the frequency of *BB+Bb* and the expression of the *B* allele were greater in the hypercalciuric stone formers than in the normocalciuric stone formers ( $p = 0.015$  and  $p = 0.018$ , respectively).

### Genotype and allele frequencies for *Apal*

Table 5 discloses no significant differences between the percent prevalence of the VDR-*Apal* genotypes and allele frequencies for the infants of each group ( $p = 0.222$ ), nor for allelic frequencies ( $p = 0.318$ ). Furthermore, hypercalciuric infants with urolithiasis had distribution of the *Apal* genotype similar to that of normocalciuric infants (Table 6) ( $p = 0.077$ ), but the expression of the *A* allele is significantly greater in hypercalciuric stone formers ( $p = 0.036$ ).

### Genotype and allele frequencies for *TaqI*

As seen in Table 5, the VDR-*TaqI* genotype differs significantly between the study and control groups ( $p = 0.043$ ); therefore, we performed further analyses by subdividing the two groups. The *TT+tt* genotype was greater than the *Tt* genotype in the study group ( $p = 0.023$ ), but no significant difference arose in allelic frequencies between the infants with urolithiasis and the

**Table 5.** Comparison of the VDR *BsmI*, *Apal*, and *TaqI* genotype distributions between the stone forming children and the controls.

	Study group (n, %)	Control group (n, %)	p
<i>BsmI</i>			
<i>BB</i>	21/72 (29.2)	16/53 (30.2)	<b>0.001</b>
<i>Bb</i>	35/72 (48.6)	37/53 (69.8)	
<i>bb</i>	16/72 (22.2)	0/53 (0)	
<i>B/b</i>	77/67	69/37	
<i>Apal</i>			
<i>AA</i>	24/78 (30.8)	11/60 (18.3)	0.222
<i>Aa</i>	42/78 (53.8)	40/60 (66.7)	
<i>aa</i>	12/78 (15.4)	9/60 (15)	
<i>A/a</i>	90/66	62/58	
<i>TaqI</i>			
<i>TT</i>	25/78 (32.1)	14/60 (23.3)	<b>0.043</b>
<i>Tt</i>	41/78 (52.6)	43/60 (71.7)	
<i>tt</i>	12/78 (15.4)	3/60 (5)	
<i>T/t</i>	91/65	71/49	

Statistical analysis: Pearson's chi-square test. *p* value  $< 0.05$  was considered as significant.

**Table 6.** Comparison of the VDR BsmI, Apal, and, TaqI genotype distributions between the hypercalciuric and normocalciuric stone formers.

	Hypercalciuric stone formers (n, %)	Normocalciuric stone formers (n, %)	<i>p</i>
BsmI			
BB	7/16 (43.8)	14/54 (25.9)	0.007
Bb	9/16 (56.3)	24/54 (44.4)	
bb	0/16 (0)	16/54 (29.6)	
B/b	23/9	52/56	
Apal			
AA	8/17 (47.1)	16/59 (27.1)	0.077
Aa	9/17 (52.9)	31/59 (52.5)	
aa	0/17 (0)	12/59 (20.3)	
A/a	25/9	63/55	
TaqI			
TT	2/17 (11.8)	23/59 (39.0)	0.102
Tt	11/17 (64.7)	28/59 (47.5)	
tt	4/17 (23.5)	8/59 (13.6)	
T/t	15/19	74/44	

Statistical analysis: Pearson's chi-square test.  
*p* value <0.05 was considered as significant.

controls ( $p=0.889$ ). In the hypercalciuric infants with urolithiasis, the distribution of the TaqI genotype and allelic distribution was similar to the infants with normocalciuric urolithiasis, as evident in Table 6 ( $p=0.102$  vs.  $0.052$ , respectively).

A comparison of infants according to stone size (i.e. <3 mm and  $\geq 3$  mm) showed similar polymorphism distributions ( $p > 0.05$ ).

## Discussion

Genetic factors are presumed to be an important risk for the development of urolithiasis, as demonstrated by the evidence of positive family history. Susceptibility genes for stone disease are the VDR gene, calcitonin receptor gene, interleukin 1 gene cluster, urokinase gene, arginine p21, VEGF, AR&ER receptor, and e-cadherin gene.<sup>17</sup> The most popular is the VDR gene, which is 5.6 kb in size and is located on chromosome 12q12–14. VDR is related to genomic and non-genomic effects of 1.25(OH)D on renal tubular cells and modulates citrate metabolism and transport of calcium and phosphate.<sup>18</sup> Allelic variations are hypothesized to affect translational efficiency or stability in messenger RNA that may result in changes in VDR expression and further changes in target genes.<sup>19</sup> For this reason, several polymorphisms of the VDR gene were evaluated, especially four single nucleotide polymorphisms (SNP): Apal, BsmI, TaqI, and Fox I. The SNPs evaluated in this study are Apal, BsmI, and TaqI. Apal and BsmI are located between the 8 and 9 exons, in the 3' region of the VDR gene; TaqI is located on the 3' noncoding sequence in exon 9. These polymorphisms of the VDR gene cause no amino acid

change in the protein, but they have strong linkage disequilibrium. Linkage disequilibrium is the non-random association of alleles at linked loci and is required to assess genes predisposition to complex diseases, such as urolithiasis.<sup>17,20,21</sup>

Many studies have shown that VDR gene polymorphism has an undeniable relationship with urolithiasis and hypercalciuria. Hypercalciuria may result from increased calcium absorption and decreased renal calcium or phosphorus reabsorption and/or enhanced bone demineralization. The VDR overexpression in the intestinal system of genetic hypercalciuric rats and duodenum and kidney cortex of genetic hypercalciuric stone forming rats was demonstrated in experimental data.<sup>13,22</sup> Also a link between idiopathic calcium stone formation and the microsatellite marker D12S339 (near the VDR locus) has been proved in humans.<sup>14</sup> In one meta-analysis, which included Asians and Caucasians, Apal and TaqI were found to be related to urolithiasis susceptibility, and hypercalciuric patients who harbored the b allele had increased risk of urolithiasis.<sup>20</sup> The other meta-analysis found a significant association of TaqI polymorphisms with urolithiasis risk.<sup>23</sup> Much research has been performed with pediatric or adult populations, but the power of our study is in selecting the first year of life, a spatial age group which has unique characteristics.

The present study revealed a significant difference in the distribution of BsmI and TaqI VDR genotypes between the stone formers and the controls ( $p=0.001$  and  $p=0.043$ , respectively). Also, the distribution of the BsmI genotype was significantly different in hypercalciuric stone formers when compared with normocalciuric stone formers ( $p=0.007$ ). Allelic frequencies were similar between the urolithiasis and control groups ( $p>0.05$ ). The B allele of BsmI and the A allele of Apal were found more prevalent in stone formers with hypercalciuria than in normocalciuric stone formers ( $p=0.018$  vs.  $0.036$ , respectively).

We found that the bb genotype is related to urolithiasis susceptibility in the infancy period, but interestingly, the expression of the bb allele was significantly higher in normocalciuric stone formers compared with hypercalciuric stone formers. We thought that having a bb allele could be an independent risk factor for stone formation in normocalciuric infants, but in other studies from Turkey, Gunes et al.<sup>21</sup> and Özkaya et al.<sup>24</sup> found no significant association between BsmI VDR polymorphism and stone formation, nor did Relan et al.<sup>9</sup>

In this study, TaqI distribution differed significantly between the study and control groups, but Apal did not. In contrast, Özkaya et al.<sup>24</sup> and Seyhan et al.<sup>25</sup> found that the frequency of the Apal AA genotype was significantly higher in the children with calcium nephrolithiasis than

in their controls. Ozkaya presented results showing that the distribution of Taql genotypes of patients with urolithiasis was similar to that of the control group, but Seyhan showed that the Taql tt genotype was significantly greater in recurrent calcium stone formers.<sup>24,25</sup> Gunes found no significant association of Taql and Apal with urolithiasis.<sup>21</sup> Nishijima et al.<sup>26</sup> reported results somewhat similar to ours, finding that Taql TT and tt genotypes are significantly higher in stone formers, and that the Apal genotype is similar between the controls and stone formers.

The diversity of these results may be due to racial genotype variability, complexity of urolithiasis etiology, sample size, differences in population characteristics and relation with environment, and gene–gene and gene–environment interactions. As mentioned above, polymorphisms on the VDR gene does not cause a particular amino acid sequence. Ebihara et al.<sup>27</sup> performed a study on a novel rat population which retains the intron 8 of VDR during alternative splicing, and showed that this event resulted in negative modulation in the vitamin D signaling pathway through a set of target genes. That the same type of change could occur in the human VDR gene is highly probable, and the relationship between VDR gene polymorphisms and phenotypes could be explained by the differences in the intron.

This study also investigated the daily vitamin D consumption and metabolic risk factors for urolithiasis. Turkey's health policy is daily administration of 400 units of vitamin D during the infancy period. Dosage of vitamin D was slightly higher in the study group, but their serum 25OHD levels were similar to that of the controls, perhaps because of the 10 premature infants among the 104 in the study group. In the study group, eight infants had hypercalcemia, but vitamin D level was elevated only in one of these, showing that vitamin D sensitivity differs in infants, and genetic differences on the receptor may play an important role in stone formation.

There are some limitations of this study. One is the limited number of participants. The other is the absence of calculus analysis. Also the study population included premature infants. This study was conducted in Turkish infants, so the results should be interpreted carefully for the other ethnicity as this study assessed the genetic predisposition to urolithiasis.

In contrast to adults, metabolic abnormalities are more associated with urolithiasis in childhood: more than 40% of all children with urolithiasis have identifiable metabolic abnormalities.<sup>6</sup> This study found that 38.5% of the infants with urolithiasis have some metabolic abnormality, as follows: 20% had hypercalciuria, 9.6% had hypocitraturia, 6.7% had hyperuricosuria, and

1.9% had hyperoxaluria. By way of comparison, in infantile cases, hypercalciuria was detected in 48% in Baştuğ's study,<sup>1</sup> 25% in Güven's study,<sup>2</sup> and 30.8% in Alpay's study.<sup>8</sup>

In conclusion, Bsm1 and Taql polymorphisms seem to be candidate genetic markers in cases of infantile urolithiasis. Identification of susceptible genes may enable determination of high-risk patients and their families, which may then allow for early diagnosis and treatment. This is the first study concerning VDR gene polymorphisms in the first year of life. Further studies should be done in order to gain a better understanding of the dynamics of infantile urolithiasis and the relationship of VDR polymorphisms with stone formation.

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### Disclosure statement

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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