Urol Res (2012) 40:35–40
DOI 10.1007/s00240-011-0399-1

ORIGINAL PAPER

Coding region analysis of vitamin D receptor gene and its association with active calcium stone disease

Abbas Basiri · Nasser Shakhssalim · Massoud Houshmand · Amir H. Kashi · Mohaddeseh Azadvari · Banafsheh Golestan · Esmaeel Mohammadi Pargoo · Hamid Pakmanesh

Received: 7 February 2011 / Accepted: 23 June 2011 / Published online: 4 August 2011

Abstract The purpose of this study was to evaluate the impact of vitamin D receptor (VDR) gene polymorphisms on the status of active renal calcium stone formation. Male active renal calcium stone formers (ASF, final $N = 106$) with two episodes of stone relapse in the past 5 years were enrolled from December 2008 to April 2009. Controls ($N = 109$) were selected from age range- and gender-matched individuals who had no evidence or history of stone disease. Sequencing and single-strand conformational polymorphism were used to determine VDR polymorphisms in the patients and controls. Three polymorphisms were identified in the VDR gene: (1) start codon polymorphism (rs2228570T>C; p.M1T); (2) C/T polymorphism in the second intron (NT-029419.12: g.10416049C>T); (3) a silent polymorphism in exon 9 (rs731236T>C; p.I352I). Start codon polymorphism was the only one that was associated with the status of calcium stone formation ($p < 0.05$). We performed a complete coding genome analysis of VDR gene and observed that only start codon polymorphism was related to the status of active calcium stone formation.

Keywords Vitamin D receptor · Polymorphism · Nephrolithiasis · Etiology · Calcium stone

A. Basiri · N. Shakhssalim · A. H. Kashi · M. Azadvari · H. Pakmanesh
Urology and Nephrology Research Center (UNRC), Shahid Labbafinejad Medical Center, Shahid Beheshti University, M.C. (SBMU), Tehran, Islamic Republic of Iran

N. Shakhssalim
UNRC, Shahid Labbafinejad Medical Center, SBMU, Tehran, Islamic Republic of Iran

M. Houshmand (✉)
Houshmand Medical Molecular Genetic Lab., Genetic Diagnostic Department, National Institute for Genetic Engineering and Biotechnology, Special Medical Center(Charity Foundation for Special Diseases), UNRC, No#103, Boostan 9th St., Pasdaran Ave., 1666677951 Tehran, Islamic Republic of Iran
e-mail: massoud_houshmand@yahoo.com

A. H. Kashi · M. Azadvari · H. Pakmanesh
UNRC, SBMU, Tehran, Islamic Republic of Iran

B. Golestan
Department of Epidemiology and Biostatistics, School of Public Health, Tehran University of Medical Science, UNRC, Tehran, Islamic Republic of Iran

E. Mohammadi Pargoo
Department of Biology, Faculty of Sciences Islamic Azad University, Science and Research Campus, Special Medical Center, Tehran, Islamic Republic of Iran

Introduction

Familial aggregation of kidney stones has been reported since 1874 [4]. Several later epidemiologic studies have emphasized a genetic predisposition to kidney stones [6, 13, 18], which is the strongest risk factor after controlling for dietary factors [5, 18]. The mode of inheritance is thought to be polygenic [2, 18].

Vitamin D is an important hormone in calcium metabolism, including intestinal calcium absorption and renal calcium resorption [7, 14]. Single nucleotide polymorphism (SNP) is one way to map complex disease traits and has been used to investigate the relationship of vitamin D receptor (VDR) polymorphisms with different diseases [2]. Vitamin D is involved in calcium homeostasis, in immune system and cancer [8]. More than 60 polymorphisms have been reported in the VDR gene. Most of these polymorphisms are in introns or regulatory areas other than exons

[20]. Some previous studies have reported a relationship between VDR polymorphisms and renal stones or hypercalciuria [2, 3, 10, 15–17], but the methods of identifying polymorphisms were insensitive and included only a small portion of the VDR gene [20]. However, it is important to analyze different polymorphisms in the VDR gene and their interrelationships to document VDR activity and function and to determine VDR haplotypes and the possibility of linkage disequilibrium in VDR polymorphisms. Determining haplotypes is important in understanding the mechanisms behind observed associations [20].

The Tehran Stone Study was a comprehensive biochemical, hormonal and genetic study in active stone formers versus control individuals. We aimed to study all VDR exons by sequencing method as a gold standard for mutation detection and to examine their association with renal stones and serum/urinary biochemistry.

Materials and methods

Patients and settings

Tehran Stone Study was a biochemical, hormonal and genetic study on active renal calcium stone formers versus control individuals. The study methods have been explained in a previous publication [19] and are summarized below. Male active stone formers (ASF), who had been referred to the Labbafinejad Urology Clinic from December 2008 to April 2009 ($N = 109$) and had agreed to participate were enrolled. ASF was defined as a patient with at least two episodes of stone relapse or increase in stone size during the past 5 years. Inclusion criteria were: male gender, age of between 30 and 55 years, absence of adrenal, hypophyseal or any systemic disease which alters calcium homeostasis, and absence of cardiovascular, gastrointestinal, hepatic or renal disease. Patients with non-calcium stones, current use of some medications [19], obesity (body mass index >30), positive urine culture and anatomical anomalies of the urinary tract were excluded from the study.

Controls were selected from patients' male colleagues or friends who were in the same age group categories. Controls were excluded if history of urinary stone disease was positive for themselves or their relatives, or if abdominal sonography revealed the presence of urinary stones. The same exclusion criteria defined for patients was applied for controls.

Blood and urine sampling

Two 24-h urine collections and a blood sample were obtained from each subject. Participants were on a free diet with no restriction of their sodium or calcium intake when they were evaluated. Urine and blood samples were used for determination of urinary and blood biochemistry and serum hormonal levels. Serum biochemistry constituted total and ionized calcium, urea, creatinine, sodium, phosphorus, chloride, potassium, uric acid, bicarbonate and PH. Urinary 24-h biochemistry constituted phosphorus, creatinine, calcium, oxalate, sodium, uric acid, magnesium, urea, chloride, citrate and potassium. The hormones investigated in this study were parathyroid hormone (PTH), 1, 25 (OH)2 vitamin D3, calcitonin, estradiol and testosterone.

Determining VDR polymorphisms

Blood was collected from patients and control individuals for isolation of genomic DNA. Total DNA was isolated by using a flexigene blood DNA kit (DNA fast, QIAGEN, Hilden, Germany) according to the manufacturer's protocol.

Eight primer pairs of PCR were designed by the Gene Runner software from the 5′ and 3′ flanking regions to amplify the exons of VDR gene (Table 1). The amplicon length varied between 300 and 700 bp.

PCR reactions were done as detailed in the following. The reaction mixture for PCR contained 200 ng DNA for amplification, 10 pmol of each primer, 1unit Taq polymerase (Sinagene, Iran), each dNTP at a final concentration of 200 μM, and 2.5 μl PCR buffer at a final volume of 25 μl.

The PCR reactions were performed in a thermal cycler (TECHNE) with the following programs: one cycle at 95°C

Table 1 Primers used for amplifying the exons of the VDR gene

Exon number	F/R	Primer sequence
Exon 2	F	5′-TGGCCCTGGCACTGACTCTG-3′
	R	5′-CCTTGCTTCTTCTCCCTCCCTTTC-3′
Exon 3	F	5′-TGGGGTGGGCCTCATGTCTTCTGT-3′
	R	5′-GGCCTTTCCCTGACTCCACTTC-3′
Exon 4	F	5′-CCCCCAACCGCAGGAGGAAGG-3′
	R	5′-TCCCCTGCCCTCTGTCCCTACTC-3′
Exon 5	F	5′-TTCCTCAAAGCCATTCCTATC-3′
	R	5′-CCTCGCCCCCGCTCCCTTACTC-3′
Exon 6	F	5′-TGGAATTCCAGTCTGGCTCTGCTG-3′
	R	5′-TTGAATTCTTGTAGCTCAGTCTAGGA-3′
Exon 7	F	5′-GTGGCTTGAAGGCGTTTACTG-3′
	R	5′-CCTTTGGTCACGTCACTG-3′
Exon 8	F	5′-GGTGGGTGGGCGGCTCCTCAG-3′
	R	5′-CTCCCTTCAGGTTGCCCAGCT-3′
Exon 9	F	5′-GGGGGTGGTGGGATTGAGCAGTGA-3′
	R	5′-CGGGGTGAGGAGGGCTGCTGAGT-3′

F forward, *R* reverse

for 5 min followed by 35 cycles at 94°C for 30 s, 55–58°C for 30 s, 72°C for 30 s and a final extension for 10 min at 72°C.

The PCR-amplified fragments were purified and sequenced by 3730XL ABI sequencer (Macrogen, Korea) using the same PCR primers. The results of DNA sequencing analysis were compared with the published revision of the National Centre for Biotechnology Information (NCBI) reference sequences using the Clustal X program. The sequence variants not matched with the corresponding records of NCBI database were defined as variation.

Screening of DNA variation in most controls was performed by PCR single-strand conformational polymorphism (SSCP) or sequencing. If a variation was observed in a VDR exon by sequencing, the SSCP band for that variation was identified and then variation in the same position of control individuals was identified by SSCP. Therefore, most cases were sequenced because we began the SSCP when we got variation through sequencing, and when variation could be confirmed by SSCP. If the SSCP could not show variation, sequencing was continued for patients and controls: for rs2228570T>C polymorphism, 85 patients were sequenced and rest of the patients (21 and 109 controls) were investigated by SSCP; for NT-029419.12: g.10416049C>T polymorphism, 78 patients were sequenced and rest of the patients (28 and 109 controls) were investigated by SSCP; for rs731236T>C polymorphism, 106 patients and 100 controls were sequenced.

For the SSCP protocol, the PCR products were diluted 1:1 by a denaturing loading buffer (95% formamide, 0.25% bromophenol blue, 0.25% xylene cyanol, 100 mM NAOH) heated in 90°C for 10 min and immediately cooled on ice. Then 20 µl of each sample was loaded on 8% polyacrylamide gel for electrophoresis with 5% glycerol. Gels were run in 1× TBE buffer under 160 V for 12 h in a 4°C cold room. After that the gels were silver stained and analyzed for detecting band shifts. Figure 1 illustrates a typical SSCP gel with bands denoting two different polymorphisms observed in this study.

The ethics of this study were approved by the Ethical Committee of the Urology and Nephrology Research Centre (UNRC) and are in accordance with the Helsinki Declaration. UNRC has adopted codes of ethics to guide human experimentations. All patients were explained about the study and informed consent was obtained.

Statistical analysis

SPSS software version 16.0 (SPSS Inc., Chicago, IL) was used to enter and analyze data. The associations of VDR polymorphisms with categorical variables were investigated by chi square or fisher exact tests as appropriate. The associations with quantitative variables were investigated by analysis of variance or *t* test. The above tests were performed separately in ASF and control groups. Bonferroni's adjustment for multiple comparisons was employed to adjust α level of significance in evaluating the associations of VDR polymorphisms with serum/urinary biochemicals in ASF patients or controls. This adjustment was also employed for comparison of serum/urinary biochemicals in ASF patients with controls. The independence of VDR polymorphisms with each other was investigated by Cramer's V test. Two-sided *p* values less than 0.05 were considered to be statistically significant.

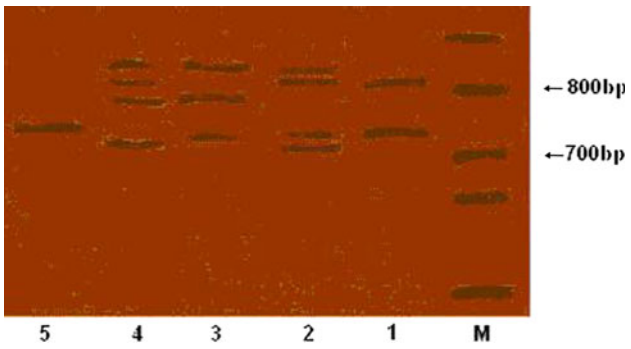

Fig. 1 PCR single-strand conformational polymorphism of VDR exon 1 in different active stone formers as explained below: *1* homozygous T>C at exon 2, *2* heterozygous T>C at exon2 and heterozygous C>T at intron 2, *3* homozygous T>C at exon2 and homozygous C>T at intron 2, *4* individual with reference alleles, M) 100 bp ladder, and *5* double-stranded PCR product

Results

A total of 106 patients and 109 controls were enrolled during the study period (3 ASF subjects were excluded from the analysis because of inadequate blood or urinary sampling). The mean ± SD of age for ASF and controls was 43.4 ± 6.9 and 38.4 ± 6.9 years, respectively ($p < 0.001$). The weight (kg) and body mass index of ASF patients versus controls were 78.4 ± 9.9 and 26.3 ± 2.7 versus 78.5 ± 10.5 and 25.8 ± 3.0, respectively (all $p > 0.05$). Details of serum and urinary biochemistry and serum hormonal levels have been previously published [19] and are summarized in Table 2.

Three polymorphisms were identified in the study population:

1. A functional T/C polymorphism was identified in the start codon (SCP) which changed methionine to threonine at the start codon. (rs2228570T>C; NT_029419.12: g.10416201T>C; p.M1T; T allele:427a.a., C allele: 424a.a.) The frequency of the C allele in ASF patients versus controls was 74% versus 47% ($p < 0.001$). The C allele was the major allele in ASF patients, while the T allele was the major allele in controls (Table 3).

Table 2 Comparison of some serum and urinary biochemicals in male active calcium stone formers and controls

Characteristic	ASF $N = 106$	Controls $N = 109$	p value[b]
Serum			
Total calcium mg/dl, mean ± SD	10.1 ± 0.6	9.9 ± 0.7	0.02[c]
Uric acid mg/dl, mean ± SD	5.3 ± 1.2	5.0 ± 1.1	0.03[c]
Creatinine mg/dl, mean ± SD	1.2 ± 0.3	1.1 ± 0.2	NS
Phosphorus mg/dl, mean ± SD	3.0 ± 0.6	3.1 ± 0.6	NS
Parathyroid hormone pg/ml, mean ± SD	48.3 ± 18.6	48.6 ± 17.6	NS[a]
Vitamin D3 pmol/l, mean ± SD	127 ± 40	93 ± 35	<0.001[a]
Calcitonin pg/ml, mean ± SD	11.6 ± 15.1	14.3 ± 15.1	NS
Estradiol pg/ml, mean ± SD	28.6 ± 6.9	30.3 ± 8.4	NS
Testosterone ng/ml, mean ± SD	5.2 ± 1.7	5.7 ± 2.1	NS[a]
Bicarbonate mmol/l, mean ± SD	55.1 ± 7.5	54.2 ± 6.9	NS
24 h urine			
Volume ml, mean ± SD	1842 ± 771	1338 ± 448	<0.001[a]
Chloride mmol, mean ± SD	197 ± 74	168 ± 52	0.004[a,c]
Calcium mg, mean ± SD	221 ± 110	156 ± 73	<0.001[a]
Oxalate mmol, mean ± SD	0.46 ± 0.19	0.34 ± 0.13	<0.001[a]
Uric acid mg, mean ± SD	464 ± 239	352 ± 206	0.001[a]
Phosphorus g, mean ± SD	063 ± 0.26	0.59 ± 0.23	NS
Sodium mEq, mean ± SD	221 ± 89	212 ± 70	NS[a]
Potassium mEq, mean ± SD	47.7 ± 20.3	53.9 ± 21.6	0.03
Citrate mg, mean ± SD	539 ± 285	411 ± 179	0.001[a]
Magnesium mg, mean ± SD	98 ± 49	91 ± 44	NS
Creatinine g, mean ± SD	1.78 ± 0.60	1.64 ± 0.55	NS[a]
PH, mean ± SD	5.97 ± 0.48	5.98 ± 0.48	NS

[a] Analysis was performed on the logarithmic transformations of these variables

[b] Based on 20 comparisons for serum and urinary biochemicals between stone formers and controls (all biochemicals are not displayed), the p value should be less than 0.0025 for a difference to be statistically significant according to Bonferroni correction for multiple comparisons

[c] Difference NOT statistically significant after Bonferroni's correction for multiple comparisons

2. A C/T polymorphism was observed in the second intron (NT_029419.12: g.10416049C>T). All polymorphisms observed in this location were heterozygous. No homozygous polymorphism was observed at this location.

3. A silent T/C polymorphism was observed at exon 9 (rs731236T>C; NT_029419.12: g.10382063 T>C), which did not change translated amino acids (p.I352I).

Out of the three polymorphisms observed in this study, two polymorphisms were in VDR exons and one was in an intron. Table 3 illustrates the frequency of the observed polymorphisms in the ASF and control groups. The associations of these polymorphisms with serum and urinary biochemistry were examined (Table 4).

Discussion

We observed three polymorphisms in the exons of the VDR gene and their adjacent introns. The rs2228570 and rs731236 polymorphisms have been reported previously, according to the search we performed in the NCBI database of SNPs. The polymorphism in the second intron was not observed in the NCBI database (accessed 31.01.2011).

SCP in exon 2 was related to the status of stone formation. SCP polymorphism (known as Fok I polymorphism [20]) is the only known protein polymorphism in the VDR gene. Previous studies [20] and the results of this study reveal that this polymorphism is independent from other polymorphisms in the VDR gene. This polymorphism results in a 424 amino acid protein (three amino acids shorter than the reference 427 amino acid protein) which has more capacity for translation [1, 20]. The association of SCP polymorphism with calcium stone formation and calciuria has previously been reported in some studies [2, 3] while several others failed to observe such an association [13, 15, 16]. We could not observe any association between SCP polymorphism and serum or urinary biochemicals.

SCP polymorphism has a wide geographic and ethnic variation [2, 15, 20, 21]. In this study, SCP C allele was observed in 47% of controls and 74% of active stone formers (Table 3). The observed frequency of C allele in ASF patients was higher than the figures reported by Bid et al. in Indian patients [2] or by Mossetti et al. in Italian patients [13].

The NT_029419.12: g.10416049C>T polymorphism in the second intron was not related to the status of stone disease. This polymorphism was not associated with other polymorphisms observed in this study.

The rs731236T>C polymorphism in exon 9 (which is known as the Taq I polymorphism [11, 12]) was not

Table 3 The polymorphisms of the VDR gene in active stone formers and control participants

	ASF	Control	Odds ratio(CI 95%)	*p* value
rs2228570T>C[a]				<0.001
TT	6 (12)	43 (88)		
TC	42 (61)	27 (39)	11.1 (4.2–29.7)[c]	
CC	54 (60)	36 (40)	10.7 (4.1–27.9)[c]	
T/C allele frequencies	54/150	113/99		<0.001
NT-029419.12: g.10416049C>T[b]			1.8 (0.8–4.0)	NS
CT	19 (61)	12 (39)		
CC	83 (47)	95 (53)		
rs731236T>C[a]				NS
TT	41 (44)	52 (56)		
TC	50 (57)	37 (43)	1.7 (0.9–3.1)[c]	
CC	11 (41)	17 (59)	0.8 (0.3–1.9)[c]	
T/C allele frequencies	132/72	141/71		NS

[a] Genetic study was successful in 102 ASFs and 106 controls

[b] Genetic study was successful in 102 ASFs and 107 controls

[c] Odds ratios were calculated compared to reference alleles (TT)

Table 4 Relationship of the VDR gene polymorphisms with selected urinary or serum biochemicals

Polymorphism	Group	Biochemistry, mean ± SD	Homozygous	Heterozygous	Reference	*p* value[c]
rs2228570T>C	ASF	Serum calcitonin, pg/ml[a]	9.3 ± 8.4	13.0 ± 20.2	27.2 ± 17.4	NS[b]
C/T intron 2[d]	ASF	Urinary 24-h phosphorus, g		0.52 ± 0.2	0.65 ± 0.3	0.048[c]
	ASF	Urinary 24-h potassium, mEq		38 ± 12	48 ± 19	0.023[c]
rs731236T>C	ASF	Urinary 24-h uric acid, mg[a]	451 ± 327	510 ± 238	396 ± 205	NS
	ASF	Urinary supersaturation of uric acid[a]	0.4 ± 0.57	0.67 ± 0.63	0.4 ± 0.37	0.005[c]
	Control	Serum bicarbonate, mmol/l	55.3 ± 7.6	51.9 ± 6.0	55.5 ± 6.9	0.034[c]
	Control	Serum parathyroid hormone, pg/ml[a]	49.9 ± 17.5	43.5 ± 14.4	52.9 ± 18.7	0.027[c]

Data are presented as mean ± SD

[a] Analysis was performed on the logarithmic transformation of these variables but for presentation in this table, actual values are presented

[b] Comparison of TT patients with TC + CC patients results in statistical significance ($p = 0.02$)

[c] As there were 20 comparisons performed for each polymorphism in ASF or control groups with serum/urinary biochemicals, the α level for statistically significant judgment must be reduced to 0.0025 according to Bonferroni's correction for multiple comparisons. Therefore, none of the *p* values in this table is statistically significant taking Bonferroni's correction into consideration

[d] NT-029419.12: g.10416049C>T

associated with the subjects' status of stone disease. The urinary supersaturation of uric acid was similar in ASF patients with homozygous CC or TT alleles of this polymorphism, but was 67% higher in subjects with heterozygous alleles (TC) (Table 4). However, this considerable difference was not statistically significant after Bonferroni's correction for multiple comparisons due to small sample size. The association of this polymorphism with urinary supersaturation of uric acid can be a risk factor for calcium stone formation, as uric acid crystals can act as a nidus for calcium stone formation [9, 13]. However, as this polymorphism is silent and does not result in amino acid change, finding the association of this polymorphism with other polymorphisms of the VDR gene, its promoter or 3′ UTR can provide a rationale for understanding the reason why this polymorphism is associated with the status of stone disease [20]. This polymorphism was not related to other polymorphisms of the VDR gene reported here. Mossetti et al. [13] reported an investigation on the relationship of Taq I polymorphism and calcium stone disease and urinary biochemistry in idiopathic hypercalciuric patients and controls. They reported a relationship between Taq I polymorphism and less urinary citrate excretion, higher saturation for calcium oxalate stone, younger age in the first episode of renal stone and a higher family score of renal stone disease but, like this study, the distribution of alleles was not different between stone formers and controls. Also Jackman et al. [10] reported the association of

Taq I polymorphism with family history score of stone disease. In these studies, the authors did not report that they had considered Bonferroni's adjustment for multiple comparisons. The frequency of C allele in the current study is around 35%, while in the Mossetti et al. study it was around 56%. We could not observe any relationship between this polymorphism and age of first renal stone episode, urinary citrate excretion or saturation of calcium (Table 4).

We admit that the sample size of this study is not large. The reason why we could hardly increase our sample size was that we employed whole coding genome sequencing as a most accurate method for detecting any polymorphism in the VDR gene (in comparison with RFLP, which is used in most published reports). VDR whole coding genome sequencing proved to be highly costly. The reason for enrolling 30–55 years old men in this study was that as the sample size was limited, we restricted the study population to a homogenous group of patients with the highest possibility of suffering from renal stones. Furthermore, we expected to receive a higher response rate from this group.

In considering the results of the current study, one should note that however we adjusted the overall possibility of type I error (α) when we compared serum/urinary biochemicals with reported polymorphisms in ASF patients or controls after Bonferroni's correction, but on the other hand, due to small sample size within ASF or control groups, the possibility of type II error increased. Therefore, other studies with higher sample size may find differences not statistically significant in this article as significant.

Conclusions

We performed a comprehensive coding region analysis of vitamin D receptor and their adjacent introns by sequencing method and observed that only start codon polymorphism was associated with the status of active calcium stone formation.

Acknowledgments This study was supported by grants from the Iranian Special Medical Centre (Charity Foundation for Special Diseases) and the Urology and Nephrology Research Centre (UNRC).

References

1. Arai H, Miyamoto K, Taketani Y, Yamamoto H, Iemori Y, Morita K, Tonai T, Nishisho T, Mori S, Takeda E (1997) A vitamin D receptor gene polymorphism in the translation initiation codon: effect on protein activity and relation to bone mineral density in Japanese women. J Bone Miner Res 12:915–921
2. Bid HK, Chaudhary H, Mittal RD (2005) Association of vitamin-D and calcitonin receptor gene polymorphism in paediatric nephrolithiasis. Pediatr Nephrol 20:773–776
3. Chen WC, Chen HY, Lu HF, Hsu CD, Tsai FJ (2001) Association of the vitamin D receptor gene start codon Fok I polymorphism with calcium oxalate stone disease. BJU Int 87:168–171
4. Clubbe W (1874) Family disposition to urinary concretions. Lancet 1:823
5. Curhan GC, Willett WC, Rimm EB, Stampfer MJ (1997) Family history and risk of kidney stones. J Am Soc Nephrol 8:1568–1573
6. Griffin DG (2004) A review of the heritability of idiopathic nephrolithiasis. J Clin Pathol 57:793–796
7. Harvey JA, Zobitz MM, Pak CY (1989) Bioavailability of citrate from two different preparations of potassium citrate. J Clin Pharmacol 29:338–341
8. Haussler MR, Whitfield GK, Haussler CA, Hsieh JC, Thompson PD, Selznick SH, Dominguez CE, Jurutka PW (1998) The nuclear vitamin D receptor: biological and molecular regulatory properties revealed. J Bone Miner Res 13:325–349
9. Hess BT, Tiselius HG (2002) Physical–chemical process in kidney stone formation. In: Coe FL, Flavus MG (eds) Disorders of bone and mineral metabolism. Lippincott Williams & Wilkins, Philadelphia, pp 619–628
10. Jackman SV, Kibel AS, Ovuworie CA, Moore RG, Kavoussi LR, Jarrett TW (1999) Familial calcium stone disease: TaqI polymorphism and the vitamin D receptor. J Endourol Endourol Soc 13:313–316
11. Konwar R, Chattopadhyay N, Bid HK (2008) Genetic polymorphism and pathogenesis of benign prostatic hyperplasia. BJU Int 102:536–544
12. Li S, Yang MH, Zeng CA, Wu WL, Huang XF, Ji Y, Zeng JQ (2008) Association of vitamin D receptor gene polymorphisms in Chinese patients with generalized aggressive periodontitis. J Periodontal Res 43:360–363
13. Mossetti G, Rendina D, Viceconti R, Manno G, Guadagno V, Strazzullo P, Nunziata V (2004) The relationship of 3′ vitamin D receptor haplotypes to urinary supersaturation of calcium oxalate salts and to age at onset and familial prevalence of nephrolithiasis. Nephrol Dial Transplant 19:2259–2265
14. Parks JH, Coe FL (1986) A urinary calcium-citrate index for the evaluation of nephrolithiasis. Kidney Int 30:85–90
15. Relan V, Khullar M, Singh SK, Sharma SK (2004) Association of vitamin D receptor genotypes with calcium excretion in nephrolithiatic subjects in northern India. Urol Res 32:236–240
16. Rendina D, Mossetti G, Viceconti R, Sorrentino M, Castaldo R, Manno G, Guadagno V, Strazzullo P, Nunziata V (2004) Association between vitamin D receptor gene polymorphisms and fasting idiopathic hypercalciuria in recurrent stone-forming patients. Urology 64:833–838
17. Ruggiero M, Pacini S, Amato M, Aterini S, Chiarugi V (1999) Association between vitamin D receptor gene polymorphism and nephrolithiasis. Miner Electrolyte Metab 25:185–190
18. Scott P, Ouimet D, Valiquette L, Guay G, Proulx Y, Trouve ML, Gagnon B, Bonnardeaux A (1999) Suggestive evidence for a susceptibility gene near the vitamin D receptor locus in idiopathic calcium stone formation. J Am Soc Nephrol 10:1007–1013
19. Shakhssalim N, Gilani KR, Parvin M, Torbati PM, Kashi AH, Azadvari M, Golestan B, Basiri A (2011) An assessment of parathyroid hormone, calcitonin, 1,25 (OH)2 vitamin D3, estradiol and testosterone in men with active calcium stone disease and evaluation of its biochemical risk factors. Urol Res 39:1–7
20. 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
21. Zmuda JM, Cauley JA, Ferrell RE (2000) Molecular epidemiology of vitamin D receptor gene variants. Epidemiol Rev 22:203–217