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ORIGINAL PAPER

Association of vitamin D receptor gene polymorphism and calcium urolithiasis in the Chinese Han population

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Abstract To investigate the effect of the vitamin D receptor (VDR) Fok I Bsm I Dde I Apa I Taq I polymorphism on the clinical presentation of calcium urolithiasis, 464 patients with urolithiasis and 450 age- and sex-matched healthy controls were recruited from The First Affiliated Hospital of Zhejiang University between January 2010 and March 2011. Five SNPs of VDR polymorphism were detected using polymerase chain reaction-based restriction analysis. The frequency of VDR Apa I genotypes between the patients and the healthy controls was significantly different ($P = 0.006$). Apa I a allele was found to be associated with increased risk of stone recurrence ($P = 0.028$). We also found Fok I Dde I Apa I showed a significant difference between male and female in the patients group ($P < 0.05$). Haplotype analysis of the five VDR polymorphisms showed a significant association with urolithiasis (global-P value $= 0.0001$). Genetic polymorphisms of VDR are important in the clinical presentation of patients with calcium urolithiasis in the Han population of southern China.

Keywords Vitamin D receptor · Calcium urolithiasis · Single nucleotide polymorphism

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Introduction

Urolithiasis is a global, multifactorial disease, the prevalence of which varies according to differences in geographical environments. In developed countries, the prevalence rate of urolithiasis is 4–20% [1]. The tendency of stone formation is largely attributed to excessive calcium absorption, since calcium is the principal crystalline constituent in upto 80% of kidney stones [2]. The cause of calcium oxalate stones is heterogeneous and might involve environmental influences, hormonal and genetic factors [3]. Besides the dietary factors, a positive family history is considered the most important risk factor to urolithiasis [4]. A growing amount of epidemiologic studies have shown familial clustering of idiopathic calcium nephrolithiasis passage. It implies that genetic factors played a significant role in the stone formation. The genetic causes have been studied extensively, whereas the genetic basis for the phenotype stills an enigmatic problem for urologist. The sole conclusion through genetic studies is that the urolithiasis is polygenic disease [5]. Another notable conclusive evidence is that calcium stone formation partly relates to the excretion of excessive amounts of calcium in the urine, or idiopathic hypercalciuria [6]. The supersaturation of urine with calcium salts depends on the urinary calcium concentration, i.e., on the urine volume and the urinary calcium excretion rate [7]. Vitamin D has an important role in calcium metabolism. It can increase the risk of urinary stone formation through the increase of serum calcium levels. Vitamin D-related effects are modulated by the vitamin D receptor (VDR). There is a evidence that the alterations in calcium transport may be caused by a pathologic increase in VDR in intestine, kidney [8], and in bone [9], so any alteration in the VDR may change the calcium metabolism, thus consequently be associated with altered

urolithiasis risk. A most recent meta-analysis suggested that allelic variations in the VDR gene were suspected to be the etiology of urinary stone disease [10]. The study of genetic polymorphisms, especially single nucleotide polymorphism (SNP), has come into view as a tool for identifying genes associated with many diseases [11, 12]. The SNP of VDR was hypothesized to influence the expression and/or function of the VDR protein [13–15]. There is limited information on the association between the VDR gene polymorphism and the development of urinary stones in Chinese Han population. Thus, we conducted a investigation on the association between the VDR gene and urolithiasis among a Chinese Han population. Five SNPs Fok I(rs2228570), Apa I(rs7975232), Taq I(rs731236), Bsm I(rs1544410) and Dde I(rs3782905) were genotyped and statistically analyzed in a case–control study including 914 Chinese individuals.

Materials and methods

Subjects

Between January 2010 and March 2011, 464 patients (aged 20–80 years) with upper urinary tract stones treated at The First Affiliated Hospital of Zhejiang University were recruited for the study. All subjects were of Chinese Han ethnic origin living in the same urban area. This group form 99% of the local population of Zhejiang, a Southeastern coastal province of China. Of note, the ethnicity of the group was also evident from the similar lifestyle and dietary habits. Any patients with the presence of kidney stone secondary to all known causes (e.g. chronic UTI, renal failure, chronic diarrhea, renal tubular acidosis, primary and secondary hyperparathyroidism, gout, cancer, and osteoporosis) were excluded. And those patients with the history of medications that affect urinary calcium excretion (e.g. diuretics) or intake of vitamin D or calcium supplements were also excluded. Diagnosis of upper urinary tract stone was confirmed by radiography of kidney-ureter-bladder, abdominal ultrasound examination or computed tomography. No patients had radiolucent stones on X-ray or had suspected cystine or uric acid stones by clinical evaluation. Thus, we considered all the stones in our patient group were calcium containing. Family history and recurrent history of urolithiasis were sought from each patient. The control group contained 450 subjects were age- and gender-matched unrelated individuals with no history of kidney stone recruited from the population at large in same area. The study was approved by local Ethical Committee, and all the patients provided informed consent before enrollment in the study.

Deoxyribonucleic acid (DNA) samples

Blood samples were obtained for genotyping of VDR polymorphisms and genomic DNA was extracted from peripheral blood leukocytes using the AxyPrep Blood Genomic DNA Miniprep Kit (Axygen Scientific, Inc, USA) according to the manufacturer's directions.

Genotyping of VDR SNPs

In our study, we tested five polymorphisms of VDR gene, which maps to chromosome 12q13.11, consists of 9 exons with at least 6 isoforms of exon 1, spans 63.5 kb, and encodes a 427-amino acid protein [16]. The genotypes of VDR SNPs were analyzed by the polymerase chain reaction-restriction fragment length polymorphism (PCR–RFLP) method. The regions containing SNPs of the VDR gene were partially amplified using the appropriate primer set. Oligonucleotide primers used for Apa I and Taq I RFLP were: forward primer 5′-CAG AGC ATG GAC AGG GAG CAA G-3′; reverse 5′-GCA ACT CCT CAT GGG CTG AGG TCT CA-3′; and for Fok I RFLP were: forward primer 5′-CCT GGC ACT GAC TCT GGC TCT G-3′; reverse 5′-GGC TCC CTT CAT GGA AAC ACC T-3′; for Bsm I RFLP were: forward primer 5′-GGT GGG ACT GAA GAA GCT GAA C-3′; reverse 5′-CTT TGG ACC TCA TCA CCG ACA T-3′; for Dde I RFLP were: forward primer 5′-AAG ACA TGG TGT CTG CTT CA-3′; reverse 5′-GGT TAG ATC GAT ATG TTT GA-3′. An initial PCR denaturation step was performed at 94°C for 4 min, followed by 35 cycles of denaturation at 94°C for 30 s, annealing at the melting temperature of each primer pair for 45 s and extension at 72°C for 45 s, with a final 10-min extension at 72°C. The different loci were recognized by the following restriction endonucleases (New England Biolabs NEB-China, Beijing): Fok I for C/T at 37°C in exon 2, Taq I for T/C at 65°C in exon 9, Bsm I for G/A at 65°C and Apa I for C/A at 37°C in intron 8, respectively, and Dde I for C/G at 37°C in intron 2. Genotypes were determined as AA (740 bp), Aa (740, 515, 225 bp) or aa (515,225 bp) for Apa I polymorphism; and TT (490, 245 bp), Tt (490, 290, 245,205 bp) or tt (290, 245, 205 bp) for Taq I polymorphism; and FF (270 bp), Ff (270, 207, 63 bp) or ff (207, 63 bp) for Fok I polymorphism; and BB (613 bp), Bb (613,357,256 bp), bb (357,256 bp) for Bsm I polymorphism; DD (304 bp), Dd (304,223,81 bp), dd (223,81 bp) for Dde I polymorphism. Before full-scale genotyping by PCR–RFLP, we confirmed the genotypes by direct sequencing. We randomly selected 2 samples from every genotype for sequencing, and the results were completely identical.

Statistical analysis

The genotype data of all tested SNPs were used to estimate Hardy–Weinberg equilibrium and evaluate association between SNP frequencies and disease phenotype by a web-based programs SHEsis http://analysis.bio-x.cn/myAnalysis.php [17]. Unconditional logistic regression was used to estimate odds ratios (OR) and corresponding 95% confidence intervals (95% CI) for the relationship between genotypes and urolithiasis. Chi-square tests were used to compare in all the other contingency table with an SPSS statistics package, version 17.0. The diversity of pairwise distribution in respective pairs of SNPs tested was assessed by the statistical measure of LD based upon calculating D' using Haploview 4.2 [18]. All statistical tests were two-sided and P value of <0.05 was considered to indicate statistical significance.

Results

Clinical characteristics of the 464 patients were presented in Table 1. Selected five SNPs of VDR were genotyped for the patient and control groups and analyzed for their association with kidney stone disease. For the five polymorphisms tested in this study within the VDR gene sequence, genotypic distributions were not deviated from HWE according to Pearson's goodness-of-fit Chi-square test (df_1) in both patients and controls. The frequencies of the Fok I, Dde I, Bsm I, Apa I, and Taq I genotypes and alleles in all patients with urinary stones and the controls are shown in Table 2. There was significant difference between the control and patient groups in the allelic distribution of the Fok I polymorphism ($P = 0.045$) and Apa I polymorphism ($P = 0.001$). However when the difference was adjusted by the Bonferroni method, the SNP of Fok I ($P = 0.045$) has no significance. Thus, only the A allele of Apa I in control group was remarkably higher than case group. This suggested that the minor allele of Apa I demonstrated protective effect to urolithiasis with odds ratios 0.72. No significant association was seen for allelic distribution of Dde I, Bsm I, and Taq I and the frequencies were comparable. Furthermore, analysis of the genotype of Apa I in patients with calcium urolithiasis and healthy controls revealed significant differences ($P = 0.006$). However, the other four SNPs Fok I, Dde I, Bsm I, and Taq I did not call for any association with calcium urolithiasis. Hence, among the five markers studied, only intronic SNP Apa I was standing out as a significant polymorphism with an increased risk in one group compared to the other. Genotypic and allelic P values were also calculated for Apa I site through a detailed statistical analysis (Table 3). Moreover, when the difference for the same variant was adjusted for multiple testing by the Bonferroni method, the global significant association obtained was still persisting ($P = 0.028$). We observed other two significant differences in AA ($P = 0.005$) and Aa ($P = 0.025$) individuals in this analysis, and odds ratio was also estimated for the these genotypes (OR: 2.04, 95% CI: 1.23–3.37 and OR: 1.37, 95% CI: 1.04–1.79).

Table 1 Characteristics of patients with kidney stone

Characteristics	Patients (n)	%	Controls (n)	%
Gender				
Male	279	60.13	263	58.44
Female	185	39.87	187	41.56
Age				
Mean (year)	50.01 ± 10.19		50.45 ± 11.22	
Familial history				
Yes	268	57.76		
No	196	42.24		
Recurrent history				
Yes	105	22.63		
No	359	77.37		
Position of stone				
Kidney	236	50.86		
Ureter	152	32.76		
Kidney and ureter	76	16.38		
Stone number				
Single	315	67.89		
Multiple	149	32.11		

The SNP data of the Apa I were also analyzed after dividing the patients into four groups according to whether they had a family history or recurrent history (Table 4). As a result, one significantly difference was found in the allelic distribution between recurrent history positive patients with negative patients ($P = 0.021$, OR = 1.562, 95% CI = 1.066–2.289), and another is the genotype AA of Apa I showed significantly higher effect for preventing stone formation recurrence ($P = 0.028$, OR = 0.22, 95% CI = 0.051–0.954). All these results gave the evidence of the minor alleles of Apa I perhaps could prevent the stone formation recurrent. However, no significant association was established between family history positive and negative patients.

We determined the VDR genotypic frequencies distribution in male and female stone formers separately (Table 5). We observed significant difference in ff of Fok I and dd of Dde I gene polymorphism in females only showing higher risk ($P = 0.03$, OR = 0.51, 95% CI = 0.28–0.94 and $P = 0.02$, OR = 0.19, 95% CI = 0.04–0.90) while no association was seen in female group. On contrast, it demonstrated 3.0- and 2.1-folds for aa and Aa of Apa I increase in

Table 2 Genotypic and allelic association analysis of vitamin D receptor single-nucleotide polymorphisms in the Chinese Urolithiasis study

SNPs ID	Controls (freq) ($n = 450$)	Stone (freq) ($n = 464$)	OR (95% CI)	*P* value	Genotype	Controls (freq) ($n = 450$)	Stone (freq) ($n = 464$)	*P* value
Fok I (rs2228570)					FF	125 (0.28)	150 (0.33)	0.122
F	476 (0.53)	534 (0.57)	1.00	0.045	Ff	226 (0.50)	234 (0.50)	
f	424 (0.47)	394 (0.43)	0.83 (0.69–1.00)		ff	99 (0.22)	80 (0.17)	
Dde I (rs3782905)					DD	8 (0.02)	14 (0.03)	0.241
D	135 (0.15)	165 (0.18)	1.00	0.11	Dd	119 (0.26)	137 (0.30)	
d	765 (0.85)	763 (0.82)	0.82 (0.64–1.05)		dd	323 (0.72)	313 (0.67)	
Bsm I (rs1544410)					BB	2 (0.01)	3 (0.01)	0.788
B	74 (0.08)	72 (0.08)	1.00	0.71	Bb	70 (0.15)	66 (0.14)	
b	826 (0.92)	856 (0.92)	1.07 (0.76–1.49)		bb	378 (0.84)	395 (0.85)	
Apa I (rs7975232)					AA	46 (0.10)	28 (0.07)	0.006
A	287 (0.31)	233 (0.26)	1.00	0.001	Aa	195 (0.43)	177 (0.38)	
a	613 (0.69)	695 (0.74)	1.40 (1.14–1.71)		aa	209 (0.47)	259 (0.55)	
Taq I (rs731236)					TT	414 (0.92)	430 (0.93)	0.757
T	863 (0.96)	892 (0.96)	1.00	0.80	Tt	35 (0.07)	32 (0.07)	
t	37 (0.04)	36 (0.04)	0.94 (0.59–1.50)		tt	1 (0.01)	2 (0.00)	

Table 3 Detailed statistical analysis of VDR Apa I site in the Chinese population

	Allelic analysis		Genotypic analysis				
	A	a	aa	Aa	AA	Global *P*	*P**
OR	1.00	0.72	1.00	1.37 (1.04–1.79)	2.04 (1.23–3.37)	0.006	0.03
95% CI	–	(0.58–0.87)					
P value		0.001	–	0.025	0.005		

*P** corresponds to global *P* value adjusted by *Bonferroni* correction for multiple tests

Table 4 Number of urolithiasis patients with family history and recurrent history for VDR gene polymorphism

	aa	Aa	AA	a	A
Familial history					
Yes	155	97	16	407	129
No	104	47	12	288	71
P value	1.00	0.14	0.782	0.39 1.14 (0.85–1.54)	1.00
Recurrent histroy					
OR, 95% CI		1.39 (0.90–2.12)	0.90 (0.41–1.97)		
Yes	67	36	2	170	40
No	192	141	26	525	193
P value	1.00	0.18	0.03	0.02	1.00
OR, 95% CI		0.73 (0.46–1.16)	0.22 (0.05–0.95)	1.56 (1.07–2.29)	

the risk of calcium stone disease in male group but not in female group.

Finally, the estimation the pair-wise linkage disequilibrium (LD) and haplotype block structure was managed by Haploview software package (Fig. 1). Evaluation of LD between each pair of loci has determined one block on the LD plot structure ("Block 1"), which spanning 26 kb between two SNPs (Dde I and Bsm I). It seems LD in this region is comparatively low, which is perhaps due to frequency of the minor alleles of these two SNPs was low so that may not

Table 5 Distribution of VDR gene polymorphism among the healthy control subjects and the urinary stone disease patients in male and female gender

VDR (Fok I) genotypes	FF	Ff	ff
Male			
Controls ($n = 263$)	70	139	54
Case ($n = 279$)	88	137	54
P, OR at 95% CI	1	0.22, 0.78 (0.53–1.16)	0.36, 0.795 (0.49–1.30)
Female			
Controls 187	55	87	45
Case 185	62	97	26
P, OR at 95% CI	1	0.96, 0.99 (0.62–1.58)	0.03, 0.51 (0.28–0.94)
VDR (Bsm I) genotypes	BB	Bb	bb
Male			
Controls	2	42	219
Case	2	45	232
P, OR at 95% CI	1	0.95, 1.07 (0.14–7.95)	0.95, 1.06 (0.15–7.59)
Female			
Controls	0	28	159
Case	1	21	163
P, OR at 95% CI	1	0.25, 0.96 (0.87–1.05)	0.32, 0.99 (0.98–1.00)
VDR (Dde I) genotypes	DD	Dd	dd
Male			
Controls	6	71	186
Case	5	78	196
P, OR at 95% CI	1	0.66, 1.32 (0.39–4.51)	0.70, 1.27 (0.38–4.21)
Female			
Controls	2	48	137
Case	9	59	117
P, OR at 95% CI	1	0.09, 0.27 (0.06–1.33)	0.02, 0.19 (0.04–0.90)
VDR (Apa I) genotypes	AA	Aa	aa
Male			
Controls	30	117	116
Case	13	109	156
P, OR at 95% CI	1	0.03, 2.15 (1.07–4.33)	0.001, 3.01 (1.55–6.21)
Female			
Controls	16	78	93
Case	14	68	103
P, OR at 95% CI	1	0.99, 1.00 (0.45–2.20)	0.55, 1.27 (0.59–2.73)
VDR (Taq I) genotypes	TT	Tt	tt
Male			
Controls	236	26	1
Case	256	22	1
P, OR at 95% CI	1	0.41, 0.78 (0.43–1.41)	0.95, 0.92 (0.06–14.82)
Female			
Controls	178	9	0
Case	174	10	1
P, OR at 95% CI	1	0.79, 1.14 (0.45–2.87)	0.31, 0.99 (0.98–1.00)

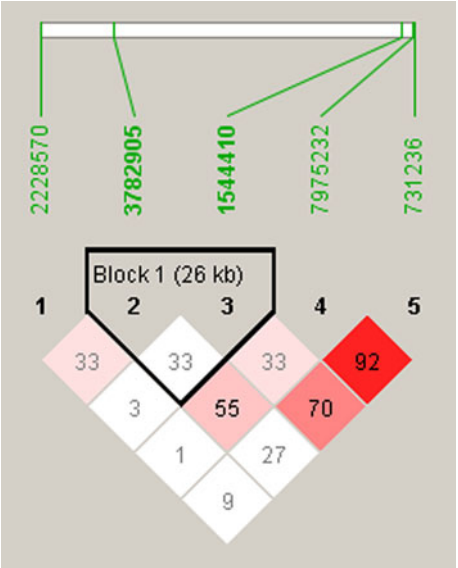

Fig. 1 Pairwise linkage disequilibrium map (LD) of VDR markers typed in this case-control study

affect the "Block 1". Whereas, more significant D' measures were found in further the 3′-terminal region between the two pairs Bsm I–Taq I and Apa I–Taq I. In addition to this result, for the detection of the closely linked genetic markers, haplotype association analysis was later conducted with the complete five-locus haplotype studied (Table 6). The analysis has shown a significant global association result with calcium urolithiasis ($\chi^2 = 25.8536$, $P = 0.0001$). As to details, the haplotype, CGGCT, CCGCT, was significantly frequent in the patient group than in the control group with a *P* value of 0.0039 (OR 1.5937 95% CI [1.1591, 2.1912]) and 0.0107 (OR 1.3150 95% CI [1.0654, 1.6230]), suggesting its association with increased disease risk. On the contrary, CCGAT haplotype was more represented in the control group than in the patient group with a *P* value of 0.0005 (OR 0.6000 95% CI [0.4483, 0.8031]), indicating its association with decreased disease risk. After Bonferroni's correction for multiple testing, the differences of CGGCT and CCGAT remained statistically significant at *P* values of 0.024 and 0.003, respectively. However, the CCGCT may be false positive for its *P* value (0.0624) exceeds 0.05 after Bonferroni's correction.

Comment

A lot of genetic epidemiological studies have been conducted to explore the relationship between SNPs and calcium urolithiasis and the results are inconclusive [19–28]. As one of the most important genes related with calcium metabolism, VDR gene has earned special concern. The VDR is a 50- to 60-kDa cellular polypeptide that belongs to the steroid-thyroid-retinoid acid receptor superfamily [29]. In addition, the human VDR gene consists of 4,605-bp complementary DNA. It is located on chromosome 12q13-14, and has 11 exons [30]. It mediates the effects of vitamin D on target cells [31]. The traditional role of vitamin D plays through $1,25(OH)_2D_3$ is in mineral metabolism, including stimulation of intestinal calcium and phosphate absorption, bone calcium and phosphate resorption, and renal calcium and phosphate reabsorption, thus facilitating the active form of vitamin D with calcium absorption and citrate excretion [14, 32]. Owing to its potent regulation activities, abnormalities of VDR have been proposed to result in kidney stone formation. Recent epidemiologic study by Zhu et al. [28] has reported that allelic variation of the VDR gene polymorphism might be associated with idiopathic hypocitraturia. It has also been reported that increase in the number of intestinal VDR was associated with increased calcium absorption in the genetic hypercalciuric rat model [8]. These findings suggest that allelic variation of the VDR gene may at least partially represent a genetic component associated with the development of urinary stones. However, the mechanism of stone formation via VDR has not been well illustrated.

The four classics single nucleotide polymorphisms (SNPs), i.e., Apa I, Bsm I, Taq I and Fok I, which were hypothesized to influence the expression and/or function of the VDR protein [13–15], had earned the focus in a search for candidate genetic markers associated with urolithiasis.

Table 6 Five-locus haplotype analysis for transmission disequilibrium test

Haplotype	Case (freq)	Control (freq)	*P* value
C C G A T	43 (0.0933)	65 (0.1437)	0.0005
C C G C T	142 (0.3054)	112 (0.2497)	0.0107
C G G C T	55 (0.1177)	35 (0.0767)	0.0039
T C G A T	42 (0.0906)	48 (0.1070)	0.2028
T C G C T	120 (0.2585)	119 (0.2648)	0.6354
T G G C T	16 (0.0339)	21 (0.0465)	0.1536
Global			0.0001

All low frequency haplotypes (<3%) were discarded from the analysis by the software SHEsis. The order of SNP: rs2228570-rs3782905-rs1544410-rs7975232-rs731236

Nevertheless, genetic epidemiological studies investigating these four urolithiasis markers yielded conflicting results. The study by Ferreira et al. [21] suggested that bb homozygous for Bsm I polymorphisms in VDR was overrepresented in hypercalciuric stone formers. Mosetti et al. [24] perceived an significant association between the Bsm I and Taq I polymorphisms and idiopathic hypocitraturia, in recurrent calcium oxalate stone disease. Nishijima et al. [25] observed that the Taq I t allele was associated with a 5.2-fold increase in the risk for severe stone disease and increased urinary calcium levels in adult Japanese patients. Mittal et al. [23] indicated that the Taq I Tt polymorphism and the Fok I Ff polymorphism are significantly associated with calcium stones in Indian people. However, there are also contrasting results have been reported. Gunes et al. [22] and Seo et al. [26] reported the VDR Apa I, Bsm I, and Taq I polymorphisms did not confer a significant risk for urinary stones in Turkish patients and Korea patients. Bid et al. [19] revealed that the Fok I polymorphism of the VDR gene was not a suitable genetic marker for urinary stone disease both in children and adult [20]. Vezzoli et al. [27] found that the Fok I genotype does not seem to be involved as a cause of idiopathic hypercalciuria and kidney stones. The analysis by Chen et al. [33] showed that the Bsm I polymorphism does not have significant difference between Taiwanese patients with calcium oxalate stones and healthy controls. These controversial results of VDR gene polymorphisms may be related to various factors, such as the small sample size from an individual study and ethnic differences associated with each study.

In the present study, allele frequencies analysis demonstrated that variant allele A of Apa I showed significantly 1.4-folds higher risk for stone formation. In addition, our results revealed that the heterozygous genotype Aa and homozygous genotype aa carrying variant allele showed significantly higher risk for stone formation. These results indicate in all likelihood that minor allele of Apa I marker is a protective allele for calcium urolithiasis in our Chinese case control studied. Gunes et al. [22] found a significant association between Apa I polymorphism and family history. Thus, we categorize the patient group to seek the relationship on Apa I polymorphism and family/recurrent history of calcium urolithiasis. The outcome reveals that in patients with recurrent urinary stone disease, the incidence of the 'a' allele was significantly higher. Furthermore, AA and Aa have significant difference between recurrent history positive and negative patients while no association was found in analysis of family history.

In our association study, we evaluated a novel VDR polymorphism detectable with Dde I restriction enzyme, which have reported a significant association in the prostate cancer [34]. However, it seems the Dde I polymorphism is not a candidate genetic marker for urinary stone disease.

It is apparent that men have higher stone incidence rates than women, implying that there could be a relationship between calcium urolithiasis among gender. We, therefore, evaluated the association of VDR genes according to gender also. From the results, we perceive that ff of Fok I and dd of Dde I show significant different in female and Aa aa of Apa I in male.

The human genome contains regions in which SNP alleles are distributed non-randomly. These regions, known as the linkage disequilibrium (LD) or haplotype blocks, have elevated local LD and limited haplotype diversity. In our study, haplotypes were inferred from the combination of five SNPs genotypes for all subjects. Through the powerful statistical analysis by software SHEsis, the frequency of the haplotype CCGAT, CGGCT of the VDR gene Fok I, Dde I, Bsm I, Apa I, and Taq I polymorphisms were significantly different in the patients with stones compared to the healthy controls. This result suggested that these haplotypes might be candidate genetic markers for urinary stone disease in Chinese Han population. Meanwhile, the three polymorphisms, Apa I, Bsm I, Taq I, have been shown to be in strong linkage disequilibrium in our results, and this results was reinforced by previous studies [14].

In conclusions, we identified a significant association between a genetic variant at the VDR locus and calcium urolithiasis in Chinese Han population. However, there have been lots of studies present pieces of evidence for the genetic baseline of stone disease; nevertheless none of them clearly demonstrate this association. So further large studies are still needed to clarify the association and explore more candidate genetic markers for urinary stone disease.

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