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ORIGINAL PAPER

Polymorphisms of the VDR gene in patients with nephrolithiasis in a Han Chinese population

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Abstract
An association study between VDR gene polymorphisms and nephrolithiasis was conducted in different populations, but it is not yet known whether the association exists in the Han Chinese population. Here, we genotyped three SNPs (rs731236, rs7975232 and rs10735810) in the promoter region of the VDR gene by iMLDR genotyping assays in a large case–control cohort. The results demonstrated that there was no association found between the three SNPs (rs731236, rs7975232 and rs10735810) in the VDR gene and nephrolithiasis, whether in allele or genotype distribution. However, SNP rs10735810 was correlated with the level of serum calcium in control groups, but not in patient groups. In conclusion, considering the large sample size, we believe that the SNP rs10735810 allele A in the VDR gene promoter region may influence the level of serum calcium, but not influence the formation of nephrolithiasis in a Han Chinese population.

Keywords VDR gene · Nephrolithiasis · Genetics

Introduction

The overall prevalence rate of nephrolithiasis was estimated to be 4.0% (4.8% in men and 3.0% in women), and the prevalence in the northern regions (4.1%) was almost the same as that in the southern regions (4.0%) in China [1]. Nephrolithiasis is a multi-factorial disease influenced by environmental and genetic factors. The genetic cause of kidney stones is complicated, ranging from the rare monogenic to the more common, polygenic forms. At least 30 genes have been reported to be associated with Mendelian forms of nephrolithiasis [2, 3], but only approximately 14% of 272 genetically unrelated individuals were shown to have the single-gene disease, as mentioned above [4]. Therefore, an in-depth investigation of susceptibility genes associated with nephrolithiasis is necessary.

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In nephrolithiasis, idiopathic hypercalciuria (IH) is an important risk factor for calcium oxalate stones [5]. More importantly, calcium oxalate is the most common crystalline component of stones. Two types of IH have been reported: absorptive hypercalciuria (AH) and renal hypercalciuria (RH). The potential mechanism of AH is through primary intestinal calcium hyperabsorption, which suppresses the secretion of the parathyroid hormone (PTH), resulting in an increase of renal filter loading [6, 7]. 1,25-Dihydroxyvitamin D3 [1,25(OH)2D3] plays an important role in the intestinal absorption of calcium and increases the serum calcium concentration [8]. 1,25(OH)2D3 exerts its activity by combining with the vitamin D (1,25-dihydroxyvitamin D3) receptor (VDR), which is a nuclear hormone receptor [9].

The VDR gene is located on 12q13.11 and encodes the nuclear hormone receptor for vitamin D3. Downstream targets of this nuclear hormone receptor are principally involved in mineral metabolism through the receptor, which regulates a variety of metabolic pathways [10]. The first evidence that connected the VDR gene to nephrolithiasis is the genetic linkage study, which comprised 54 sibships with a total of 303 pairs of siblings, concordant for greater or equal to 1 stone episode. The results demonstrated that the microsatellite marker D12S339 (near the VDR locus, $P = 0.01$), as well as two flanking markers (D12S1663: $P = 0.03$ and D12S368: $P = 0.01$), displayed linkage to nephrolithiasis

[11]. Next, more studies were conducted to investigate the associated relationship between VDR gene polymorphisms and nephrolithiasis. Most of these studies concluded that polymorphisms of the VDR gene are associated with the production of kidney stones in different populations [12–14], especially in children with nephrolithiasis [15]. However, no related investigations have been conducted in the Han Chinese population.

Considering the small sample size of previous studies and racial limitation, in this current study, we conducted research regarding the association between polymorphisms of the VDR gene and nephrolithiasis in a Han Chinese population. After detailed searching, SNPs (rs731236, rs7975232 and rs10735810) in the VDR gene promoter region were genotyped in large samples, which were calculated by scientific statistical tools (more information in the methods). These three important promoter region SNPs (rs731236, rs7975232 and rs10735810) in the VDR gene were mostly investigated in previous studies in regard to kidney stone association [16]. The aim of the present study was to evaluate the association of the VDR gene polymorphisms with nephrolithiasis.

Methods

Samples

A total of 943 patients with nephrolithiasis were recruited in the Second Affiliated Hospital of Third Military Medical University from 2013 to 2016. All patients were interviewed individually by trained doctors and were examined by spiral computed tomography (CT), ultrasonography or X-ray. Patient inclusion criteria: ① two or more calcium oxalate stones had been excreted or removed during the previous 2 years; or computed tomography (CT), ultrasonographic or X-ray radiographic evidence of stones; ② no history of medications that affect urinary calcium excretion or excessive intake of vitamin D. For the control group, 975 healthy volunteers were recruited from the community. Control inclusion criteria: ① evidence of a lifetime absence of nephrolithiasis by CT, ultrasonography or X-ray; ② no significant complication of disease of nephrolithiasis (kidney colic and hydronephrosis). All participants were Han Chinese. Family history was obtained from the proband, which included each participating parent and additional relatives as needed, by two independent raters, including a nurse who recorded pedigree information, and by the clinical interviewer, who acquired family history in detail during the clinical interview.

This study was conducted with the approval of the ethics committee of Third Military Medical University. We used the following criteria to evaluate whether the participants had the capacity to consent: first, patients had the ability to understand; second, patients had the ability to reason; and third, patients had the ability to make rational decisions. All participants gave written informed consent.

Genotyping

Peripheral blood (5 ml) was collected from all participants, and genomic DNA was isolated from peripheral blood cells using a TIANamp Blood DNA kit (TIANGEN BIOTECH, BEIJING) according to the manufacturer's instructions. Quality control of DNA was conducted by agarose gel electrophoresis and λDNA-*Hin*dIII digested bands. The SNPs (rs731236, rs7975232 and rs10735810) in the promoter region of the VDR gene were genotyped in both case and control groups, which was performed with improved multiple ligase detection reaction (iMLDR) genotyping assays with technical support from the Center for Genetic & Genomic Analysis (Genesky Biotechnologies Inc., Shanghai).

Statistical analysis

Sample size was calculated using the website (http://powerandsamplesize.com/Calculators/) [17]. The minor allele frequency (MAF) of three SNPs (rs731236, rs7975232 and rs10735810) was calculated according to the 1000 Genome program (http://www.internationalgenome.org/). The parameter setting of calculated sample size needed to test odds ratio was the following: power ($1-\beta$, 90%), Type I error (α, 0.05), odds ratio (OR, 2), P_A (rs731236, 0.05; rs7975232, 0.26; rs10735810, 0.42) and P_B (rs731236, 0.03; rs7975232, 0.22; rs10735810, 0.36). The sample sizes necessary for each of the SNPs (rs731236, rs7975232 and rs10735810) were 1666, 1831 and 1051, respectively.

Hardy–Weinberg equilibrium was calculated using Plink 1.09 [18]. Clinical characteristics are presented as the means ± SD. Wilcoxon signed-rank test and λ^2 test were used for comparison of sex, age, race, and BMI, as appropriate. Bonferroni correction was used for the analysis of contingency tables, depending on the sample size. Spearman correlations and heat map plots were conducted using R software (http://www.R-project.org/). A general linear model was employed to analyze the association between cases and controls, correcting for covariance.

Results

Demographic characters and serum parameters in patients with nephrolithiasis and control subjects

Sex and BMI (body mass index) distribution demonstrated no significant difference ($P > 0.05$, Table 1) between patients with nephrolithiasis ($n = 943$) and control subjects ($n = 975$). There was a significant difference in age between patients with nephrolithiasis (51.2 ± 14.13 years) and control subjects (54.33 ± 18.11 years, $P < 0.001$). Almost half of the subjects accepted examination of serum parameters (including serum potassium, sodium, chlorine, calcium, and phosphate) in the clinical experiment lab. The results of serum parameters are shown in Table 1, and no significant difference was found between patients with nephrolithiasis and control subjects.

Comparison of vitamin D receptor genotypes in patients with nephrolithiasis and control subjects

The genotype distribution did not deviate from Hardy–Weinberg equilibrium. The genotype and allele distributions of the 3 VDR gene polymorphisms (rs731236, rs7975232 and rs10735810) for the nephrolithiasis and control groups are shown in Table 2. No significant association was found in polymorphisms of rs731236 and rs7975232 between cases and controls, even when corrected with covariates (age, sex and BMI) (Table 2). In addition, current results suggest no association exists between nephrolithiasis and SNP rs10735810 ($P = 0.074$), even when corrected with covariates (age, sex and BMI) (Table 2, $P = 0.064$).

Haplotype analysis of three SNPs in the VDR gene in patients with nephrolithiasis and control subjects

Haplotype analysis of the three SNPs in the VDR gene was completed by Plink online software (https://www.cog-genomics.org/plink2). Multimarker haplotypes within the VDR gene promoter region in patients with nephrolithiasis and

Table 1 Comparison of clinical characteristics and serum parameters between patients with nephrolithiasis and control subjects

Characterize	Nephrolithiasis ($N = 943$)	Normal control ($N = 975$)	Wilcox/Chisq*	P value
Sex				
Male	627	614	2.44*	0.118
Female	316	361		
Age	51.2 ± 14.13 (51)	54.33 ± 18.11 (56)	395,720	**< 0.001**
BMI (kg/m^2)	24.41 ± 3.52 (24.3)	24.36 ± 10.81 (24.1)	468,250	0.095
Serum potassium	3.87 ± 0.39 (3.86)	3.89 ± 0.39 (3.88)	69,600	0.115
Serum sodium	139.71 ± 2.42 (139.9)	139.95 ± 2.36 (140.2)	69,869	0.136
Serum chlorine	105.52 ± 3.02 (105.4)	105.32 ± 2.88 (105.5)	74,696	0.946
Serum calcium (mmol/l)	2.30 ± 0.13 (2.3)	2.29 ± 0.13 (2.3)	78,866	0.518
Serum phosphate (mmol/l)	1.10 ± 0.22 (1.12)	1.11 ± 0.27 (1.08)	78,572	0.581

Significant P value is in bold ($P < 0.05$)

Value in brackets denote medium

Table 2 Prevalence of vitamin D receptor genotypes in patients with nephrolithiasis and control subjects

SNP(hg19)	Genotype/Allele	Nephrolithiasis ($N = 943$)	Normal ($N = 975$)	Chi-square test				Linear regression model	
				λ value	OR	95% CI	P value	T value	P value
rs731236	AA/GA/GG	849/92/2	870/103/2	0.34			0.84	0.176	0.86
	A/G	1790/96	1843/107	0.23	1.08	[0.82, 1.44]	0.63		
rs7975232	AA/CA/CC	65/394/484	78/417/480	1.32			0.52	− 1.32	0.188
	A/C	524/1362	573/1377	1.13	0.925	[0.80, 1.06]	0.29		
rs10735810	AA/GA/GG	238/477/228	223/482/270	3.52			0.172	1.852	0.064
	A/G	953/933	928/1022	3.2	1.13	[0.99, 1.28]	0.074		

Linear regression model was employed for analysis of the association between cases and controls correcting for age, sex and BMI

control subjects denoted no statistically significant association (Table 3).

Association analysis of SNP rs10735810 and serum parameters

Spearman correlation between SNPs (rs731236, rs7975232 and rs10735810) in the VDR gene and clinical experiment lab examination is presented in Fig. 1. A negative correlation was found between the SNP rs10735810 and the level of serum calcium ($R = -0.1$ $P = 0.003$). Further analysis of rs10735810 and serum parameters using a general linear model (corrected by age, sex and BMI) indicated an association exists between SNP rs10735810 and the levels of serum calcium in controls (Table 4, $R = -0.87$, $P = 0.00467$), but not between rs10735810 and the levels of serum calcium in cases (Table 4, $R = -0.06$, $P = 0.836$). No association was found between the other two SNPs (rs731236 and rs7975232) and serum parameters.

Table 3 Multimarker haplotype (rs731236–rs7975232–rs10735810) analysis within the VDR gene promoter region in patients with nephrolithiasis and control subject

Haplotype	Frequency in nephrolithiasis	Frequency in controls	χ^2 (1 *df*)	*P* (adjust)
AAA	0.08338	0.0854	0.04783	0.8269
ACA	0.4045	0.433	3.047	0.08091
AAG	0.1062	0.09886	0.5301	0.4666
ACG	0.406	0.3827	2.057	0.1515

Globe $\chi^2 = 3.54$; $df = 3$; $P = 0.316$

Discussion

In the present study, we found no significant association between the VDR gene polymorphisms (rs731236, rs7975232and rs10735810) in the promoter region and nephrolithiasis. Additionally, analysis of multimarker haplotypes within the promoter region in cases and controls denoted no statistically significant association with nephrolithiasis. However, SNP rs10735810 was associated with the level of serum calcium in controls, but not in patient groups using a general linear model. No association was found between

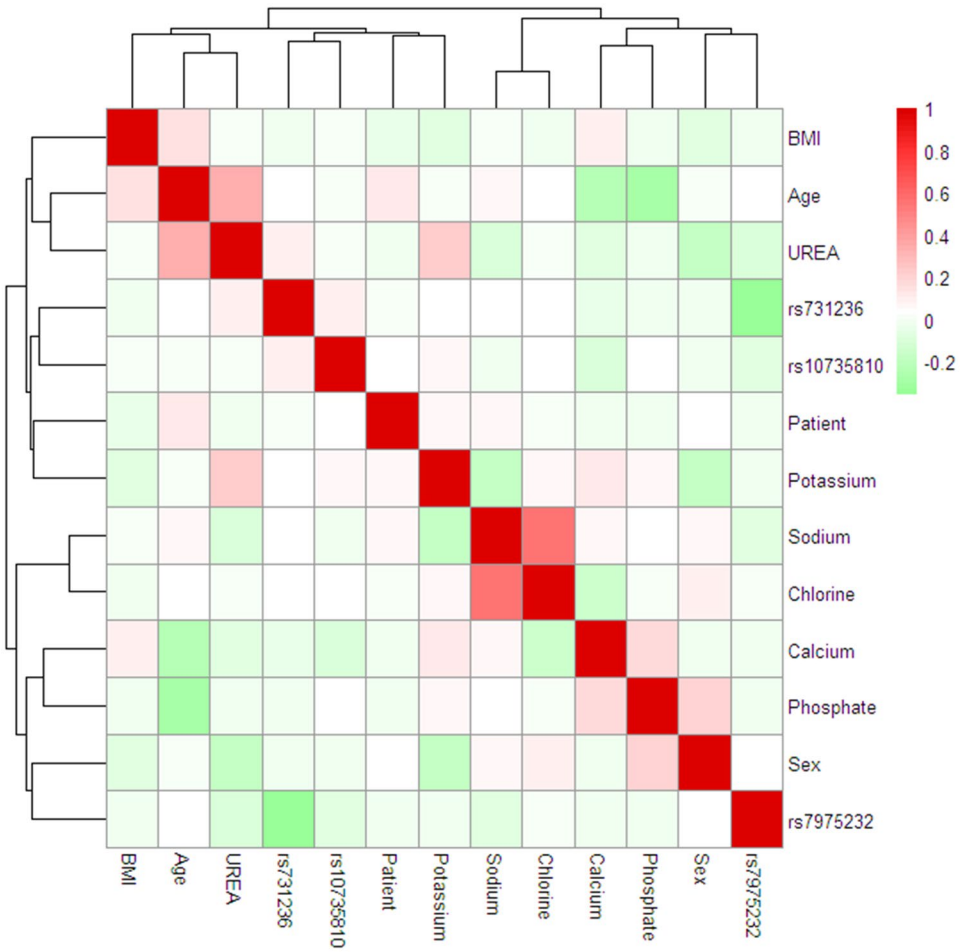

Fig. 1 Heat map plot of spearman correlation between SNPs (rs731236, rs7975232 and rs10735810) in VDR gene and clinical experiment lab examination

Table 4 Association analysis of rs10735810 and serum parameters using linear regression model (corrected by age, sex and BMI)

	Nephrolithiasis			Normal controls		
	β coefficient	T value	P value	β coefficient	T value	P value
Age	0.00248	0.954	0.3408	− 0.003511	− 1.543	0.12375
Sex	− 0.0827	− 1.044	0.2972	− 0.145031	− 1.746	0.08171
BMI	− 0.0027	− 0.28	0.7797	− 0.000501	− 0.045	0.96404
Calcium	− 0.0609	− 0.207	0.836	− 0.87185	− 2.846	**0.00467**
Phosphate	0.29977	1.759	0.0794	0.1265459	0.822	0.41185
Potassium	0.03049	0.3	0.7647	0.0887281	0.849	0.39649
Sodium	− 0.0076	− 0.401	0.6887	− 0.006761	− 0.316	0.75189
Chlorine	0.00109	0.073	0.9419	0.0084123	0.489	0.62537

Significant P value is in bold ($P < 0.05$)

the other two SNPs (rs731236 and rs7975232) and serum parameters.

Vitamin D binds to its nuclear receptor (VDR gene encoding) and regulates different biological functions, including calcium homeostasis. Calcium oxalate which consists of calcium salt and oxalate is the main chemical composition of stones. Polymorphisms in VDR gene promoter region can influence VDR mRNA expressions which further influence the function of VDR receptor. Many publications have documented the association of SNPs (rs731236, rs7975232 and rs10735810) in the VDR gene with kidney stones. However, these reports published conflicting data. SNP rs10735810, also known as rs2228570 or VDR Fok1 polymorphism, is located in the promoter region of the VDR gene (chr12:47879112).The VDR Fok1 f allele and genotype were not associated with nephrolithiasis risk in a Caucasian population [19, 20], but they were associated with nephrolithiasis risk in an Asian population [21]. In the current investigation, the SNP rs10735810A allele had a trend of enrichment in patients with nephrolithiasis ($\lambda = 3.2$, $P = 0.074$), even after correcting for the covariance of age, sex and BMI in a general linear model ($T = 1.852$, $P = 0.064$). More interesting, we found that the SNP rs10735810 A allele was associated with higher levels of serum calcium in case and control groups. Therefore, current investigation indicates rs10735810 A allele may lead to calcium accumulation in kidney tubules which further induces the formation of stones.

In addition, Nishijima et al. concluded that the rs731236G allele was associated with a 5.2-fold increase in the risk of severe stone disease in a Japanese population. The urinary calcium level in patients with the AG and GG genotypes was also higher than those with the AA genotype [22]. These results were confirmed by a recent investigation [14]. However, it has also been reported that no association was found between the rs731236 genotype in the VDR gene with kidney stones in European [23], Italian [24], Indian [12] and Turkish populations [13]. More surprisingly, Mossetti et al. demonstrated that the prevalence of AA genotypes of rs731236 was significantly higher in hypocitraturic stone former patients than in normocitraturic stone former patients and controls [25]. Likewise, Ozkaya et al. reported that the frequency of the rs7975232 AA genotype was significantly higher in children with calcium nephrolithiasis than the controls, and no association of rs731236 polymorphisms and nephrolithiasis was found.

In contrast, Söylemezolu concluded that the rs7975232AA genotype was associated with a 3.5-fold increased risk for idiopathic hypercalciuria compared with the AC/CC genotype [odds ratio 3.5, 95% confidence interval (1.1–11)], but the rs731236 polymorphism did not show any significant association with absorptive hypercalciuria. The same situation was also found in the investigation about the rs10735810 genotype. However, previous investigations were done in the condition of small sample size or none power evaluation before the study design. Therefore, a false-positive association may have occurred because of the small sample size. In the current study, we evaluated the sample size needed for the study design, which proved to be the largest study cohort until now. The results confirmed that no association was found between SNPs (rs731236 and rs7975232) in the VDR gene and patients with kidney stones.

In addition, considering the haplotype analysis of three SNPs, Rendina et al. showed that the prevalence of rs7975232 and rs154410 VDR genotypes and alleles in patients with fasting idiopathic hypercalciuria (IH) was significantly different ($P < 0.05$) from that observed in patients with absorptive IHc and control subjects, and the ba haplotype was overrepresented in these patients. Additionally, Mossetti et al. demonstrated that patients and controls were classified as homozygous (bbTT and BBtt) or heterozygous in relation to the rs154410 and rs7975232 polymorphisms [25]. Compared with BBtt patients, bbTT homozygous stone formers showed lower citrate excretion (1.91 ± 0.89 vs 3.46 ± 1.39 mmol/24 h, $P = 0.004$) and higher calcium oxalate salts (2.02 ± 0.51 vs 1.53 ± 0.53, $P = 0.006$). However, current research denoted no statistically significant

association between the haplotypes of the 3 SNPs with nephrolithiasis.

Conclusion

In conclusion, considering the large sample size, we have reason to believe that the SNP rs10735810 allele A in the VDR gene promoter region may influence the level of serum calcium, but not the formation of nephrolithiasis in a Han Chinese population.

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Compliance with ethical standards

Conflict of interest The authors declare no competing financial interest.

Ethical approval This study was conducted with the approval of the ethics committee of Third Military Medical University.

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