

Polymorphisms in the vitamin D receptor and risk of gout in Chinese Han male population

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Abstract Previous studies have showed that patients with gout showed lower serum 25(OH)D levels. As the specific receptor of vitamin D, VDR plays an important role in regulating immune system by combining with vitamin D. In this study, we investigated whether the functional *VDR* polymorphisms were associated with susceptibility to gout in Chinese Han male population. A total of 504 patients with gout and 523 gout-free controls were recruited from the Affiliated Hospital of the Medical College, Qingdao University. Genotyping of *VDR* rs11568820, rs2228570 and rs1544410 was performed by TaqMan allele discrimination assays. An association analysis was carried out using the χ^2 test. A genotype–phenotype analysis was also conducted. Our results showed that polymorphisms of rs11568820 and rs1544410 in *VDR* were associated with gout in Chinese Han male population. The A allele of both rs11568820 and rs1544410 was associated with the risk of gout [$P = 0.012$ OR 1.251, 95 % CI (1.051–1.490); $P = 0.006$, OR 1.574,

95 % CI (1.139–2.175)]. However, there was no statistic significance between rs2228570 and gout ($P = 0.186$). Our study suggested that the polymorphisms of *VDR* may be relevant host susceptibility factors for the development of gout in Chinese Han male population. However, further study should be done in a larger size sample and other ethic to test and verify our result.

Keywords Gout · VDR · Susceptibility · Chinese Han population

Introduction

Characterized by hyperuricemia, gout is a kind of polygenic disease which results from disorder of purine metabolism and/or impaired renal excretion of uric acid. Resulting from the interaction of genetic and environmental risk factors like high protein (red meat and seafood) and alcohol (mainly beer and spirits), its prevalence has recently

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increased considerably and it has become the common inflammatory arthritis in man with the syndrome of recurrent attacks of acute arthritis, deposition of monosodium urate monohydrate (MSU) in and around the joints and even uric acid nephrolithiasis [1]. The gout-associated MSU crystal can activate the NALP3 (NACHT, LRR and PYD domains-containing protein 3) inflammasome, which lead to processing of procaspase-1 to caspase-1 and the production and secretion of active IL-1 β and IL-18, and then lead to a series of inflammatory response of arthritic diseases [2]. Gout is also a wide spread metabolic disease associated with other abnormalities of metabolic syndrome such as hyperglycemia, obesity, dyslipidemia, hypertension and hyperglycemia [3–5].

Vitamin D is well known for its role in maintaining calcium homeostasis and promoting bone mineralization [6]. In addition, it affects the immune, nervous, endocrine and reproduction system, and even some scholars think it is a kind of hormone related to many metabolism diseases rather than vitamin [7]. Vitamin D deficiency can increase systemic inflammation, as documented by elevated levels of C-reactive protein (CRP) and interleukin-10 and so on. VDR (vitamin D Receptor) is the specific receptor of vitamin D located on chromosome 12q12-14, which encodes a ligand dependent transcription factor that belongs to the superfamily of nuclear hormone receptors. Up to now, several common functional polymorphisms such as rs2228570, rs1544410, rs11568820 and rs7975232 have been identified. As an important mediator of the vitamin D pathway, polymorphisms of *VDR* may have important influence on its function [8]. Recent studies have suggested that functional variants of *VDR* were associated with many diseases, including arthritic diseases [9], T1DM [10], osteoporosis [11], obesity [12] and hyperparathyroidism [13], indicating that different genotypes of *VDR* polymorphisms might play different roles in the onset of metabolism diseases. Since gout is a kind of metabolism disease related to such diseases, we suppose that *VDR* might be involved in the development of gout. Moreover, it has been reported that patients with hyperuricemia demonstrated significantly lower levels of serum vitamin D compared with those in controls and low serum levels of 25-(OH)D were associated with gout activity [14]. In our study, we chose three functional SNPs including rs2228570, rs1544410 and rs11568820 in *VDR* to examine our hypothesis. The rs11568820 in the promoter region was associated with capability of *VDR* transcription in intestinal epithelial cells. The rs2228570 in exon 2 causes an alternative transcription initiation site, resulting in a *VDR* protein with addition of three amino acids. The rs1544410 located in the intron between exon 8 and 9, next to the 3' end of the *VDR* would probably influence the mRNA stability by forming haplotype with the poly A VNTR in the 3' UTR.

In this present study, we investigated whether the single nucleotide polymorphisms (SNP) in *VDR* would contribute to clarify the relationship with gout, and thus to examine the possible genetic association between these polymorphisms of *VDR*, including rs11568820, rs2228570 and rs1544410, and gout in Chinese Han male population.

Materials and methods

Study subjects

The gout patients and gout-free controls were recruited from the Department of Gout laboratory at the Affiliated Hospital of the Medical College, Qingdao University. In total, 504 male patients (mean age 52.22 ± 13.79 years) with gout and 523 ethnically matched male controls (mean age 51.60 ± 11.99 years) were genotyped for the three SNPs of *VDR*. The diagnosis of gout was based on the preliminary criteria, which were published by the American College of Rheumatology in 1997. Meanwhile, gout-free controls without a personal or familial history of hyperuricemia or gout or other serious illness were collected. All patients and controls were selected from the same population residing in Shandong province, China. The biochemical parameters including blood glucose, uric acid, total cholesterol (TC), triglycerides (TG), urea nitrogen, creatinine in the plasma and patients' characteristics ranging from demographic data to clinical parameters (tophi- and disease-related complications) were measured or recorded. All subjects were provided written informed consent, and the study protocol was approved by the Ethics Committee of Affiliated Hospital of the Medical College, Qingdao University.

Genotyping

Genomic DNA was extracted from peripheral blood leukocytes (5 ml) using standard methods. The target region was amplified by the TaqMan allele discrimination assays with a VIC/FAM labeled probe. The segments of the three polymorphisms were replicated using the following primers: forward primer 5'ACCCATAATAAGAAATAAGTTT TTA3' and reverse primer 5'TGTGACCTAGTTTACTCAG GAATAT3' for rs11568820; forward primer 5'GGAAGT-GCTGGCCGCCATTGCCTCC3' and reverse primer 5'TC CCTGTAAGAACAGCAAGCAGGCC3' for rs2228570; and forward primer 5'GAGCAGAGCCTGAGTATTGGGA ATG3' and reverse primer 5'GCAGGCCTGTCTGTGGCCC CAGGAA3' for rs1544410. Probe and primers were both synthesized from the Life Technologies Company. Polymerase chain reaction (PCR) were carried out in a final volume of 25 μ l containing 2 \times PCR Master Mix 12.5 μ l,

Table 1 Demographic and clinical characteristics of the study population

Parameter	Gout (<i>n</i> = 504)	Normal (<i>n</i> = 523)	<i>T</i>	<i>P</i>
Age (years)	52.22 ± 13.79	51.60 ± 11.99	1.21	0.23
Tophi, <i>n</i> (%)	117 (23.7 %)	–	–	–
BMI (kg/m ²)	27.06 ± 3.45	25.44 ± 4.72	5.67	<0.01
Systolic pressure (mmHg)	137.16 ± 18.46	132.79 ± 17.86	3.34	<0.01
Diastolic pressure (mmHg)	89.75 ± 12.04	84.42 ± 9.84	6.64	<0.01
Blood glucose (mmol/L)	6.17 ± 1.72	5.73 ± 1.63	4.08	<0.01
Uric acid (μmol/L)	472.76 ± 117.18	324.36 ± 65.38	24.52	<0.01
Triglycerides (mmol/L)	2.49 ± 1.93	1.65 ± 1.22	8.15	<0.01
Total cholesterol (mmol/L)	5.39 ± 1.19	5.09 ± 1.05	4.24	<0.01
Urea nitrogen (mmol/L)	5.83 ± 2.72	5.38 ± 1.10	3.43	<0.01
Creatinine (μmol/L)	91.06 ± 31.37	84.85 ± 17.30	3.83	<0.01

20 × SNP Genotyping Assay 1.25 μl, DNA sample and DNase-free water 11.25 μl. The reaction was carried out as follows: 95 °C for 3 min, followed by 95 °C for 15 s and 60 °C 1 min for 40 cycles. After the expansion, we analyzed the genotypes combining with a scatter diagram and the amplification curve.

Statistical analysis

In this study, SPSS 17.0 software was used for a statistical analysis. The student's *t* test was used to assess a significant difference in demographic and clinical characteristics between cases and controls. Hardy–Weinberg equilibrium of the allele distribution was tested. The OR and 95 % CI were used as a measure of the strength of relationships in the genotype distribution and allele frequencies between the cases and controls, tophi patients and non-tophi patients. Pearson's χ^2 test was used to compare the genotypic and allelic frequencies between controls and patients (if expected values were below 5, Fisher's exact test was used). An analysis of variance (ANOVA) was used to calculate the association between genotypes and demographic and clinical characteristics among gout patients, including age, age at diagnosis, duration of gout history, blood pressure, body mass index (BMI), waist-to-hip ratio (WHR), tophi, hypertension, diabetes, obesity and serum biochemistry. *P* values <0.05 were regarded as statistically significant.

Results

Demographic and clinical characteristics of the study population

The clinical characteristics of the population enrolled in the study are summarized in Table 1. The results showed that

gout patients had significant higher BMI values, systolic pressure levels, diastolic pressure levels, blood glucose levels, uric acid levels, TC levels, TG levels, urea nitrogen and creatinine levels than the controls (*P* < 0.01). In addition, the mean age tended to be higher in the gout patients, but did not reach statistic significance (*P* = 0.23). About 23.7 % of the patients with gout had tophi.

Hardy–Weinberg equilibrium

In the three polymorphisms, the genotype distributions all followed the Hardy–Weinberg equilibrium among control population (*P* = 0.176 in rs1544410, *P* = 0.756 in rs2228570, *P* = 0.834 in rs11568820).

Analysis of genotypic and allelic frequency

The population involved in the study was age-matched, and the associations in genotypic and the allelic frequency between cases and gout-free controls of the three SNPs are listed in Table 2. Distributions of allelic frequency of rs11568820 and rs1544410 in *VDR* showed statistical significance (for rs11568820, *P* = 0.012, OR 1.205, 95 % CI [1.051–1.490] by allele, *P* = 0.011 by genotype; for rs1544410, *P* = 0.006, OR 1.574, 95 % CI [1.139–2.175] by allele, *P* = 0.003 by genotype). For rs11568820, a G/A polymorphism, the A allele seemed to be the risk allele for predisposition to gout (*P* = 0.018 after adjust the confounders of gout by performing a logistic regression analysis, Table 2). Similarly, for rs1544410, a G/A polymorphism, the A alleles seemed to be the risk allele for gout as well [*P* = 0.006, OR 1.574, 95 % CI (1.139–2.175); *P* = 0.014 after adjust the confounders of gout by performing a logistic regression analysis] (Table 2). However, the rs2228570 AA and AG genotypic and A allelic frequencies demonstrated no association with increased risk of gout (*P* = 0.186). Concerning tophi patients and non-tophi

Table 2 The distribution of genotypic and allelic frequency of rs11568820, rs2228570 and rs1544410 in VDR between cases and controls

(1)	(2)	(1 + 2)	(3)	(1) versus (2)	(1 + 2) versus (3)	(1 + 2) versus (3)
Tophi patients	Non-tophi patients	Gout patients	Control	<i>P</i> value	<i>P</i> value	Adjusted <i>P</i> value*
(<i>n</i> = 121)	(<i>n</i> = 384)	(<i>n</i> = 504)	(<i>n</i> = 523)	OR (95 % CI)	OR (95 % CI)	Adjusted OR (95 % CI)*
Polymorphism rs11568820						
Genotypes						
GG	30	102	182	0.921	0.011	0.018
AG	68	209	255			
AA	23	72	86			
Alleles						
A	114	353	427	0.781	0.012	1.291 (1.044–1.597)
G	128	413	619	1.042 (0.780–1.392)	1.251 (1.051–1.490)	
Polymorphism rs2228570						
Genotypes						
GG	39	123	146	0.589	0.186	0.076
AG	64	188	264			
AA	18	72	113			
Alleles						
G	142	434	556	0.580	0.069	1.206 (0.980–1.483)
A	100	332	490	1.086 (0.810–1.456)	1.175 (0.987–1.399)	
Polymorphism rs1544410						
Genotypes						
GG	101	325	460	0.610	0.003	0.014
AG	15	43	59			
AA	3	17	4			
Alleles						
A	21	77	67	0.592	0.006	1.447 (1.078–1.943)
G	217	693	979	0.871 (0.525–1.445)	1.574 (1.139–2.175)	

* The OR, 95 % CI and *P* values were estimated by a logistic regression after adjustment of age, BMI, systolic pressure and diastolic pressure

patients, the distributions of the three SNPs were all not crucial risk factors for the occurrence of tophi [$P = 0.069$, OR 1.175, 95 % CI (0.987–1.399)]. Moreover, the analysis of linkage disequilibrium between rs11568820 and rs1544410 was done further, and the D value was 0.147, while the r^2 value was 0.002, which showed that there was no linkage disequilibrium between these two sites. Sites rs11568820 and rs1544410 of VDR were independent to each other.

Genotype-phenotype analysis

Since the polymorphisms of rs11568820 and rs1544410 were significant associated with gout, ANOVA was used for a genotype–phenotype analysis of the gout patients (Table 3). Significantly different genotypic distribution was found in disease duration, serum uric acid levels and serum creatinine levels ($P < 0.05$) of rs11568820 and rs1544410, and details are showed in Table 3. In addition, patients with AG genotype of rs1544410

showed higher systolic pressure levels than those with the AA or GG genotypes (144.02 ± 21.86 mmHg vs. 136.23 ± 17.79 mmHg, $P = 0.003$; 144.02 ± 21.86 mmHg vs. 132.68 ± 19.36 mmHg, $P = 0.020$). Moreover, a statistical difference was found in the genotypic frequencies of rs1544410 in diastolic pressure levels between genotype AA and GG (89.04 ± 11.37 mmHg vs. 88.32 ± 12.57 mmHg, $P = 0.045$) and in the genotypic frequencies of GG genotype vs AA and AG genotypes ($P = 0.038$).

Furthermore, as presented in Table 3, for rs11568820 and rs1544410, stratification of the age at diagnosis by genotype demonstrated different effects [15]. There were differences between the GG genotype and AA genotype ($P = 0.039$), and the AA genotype versus AG and GG genotypes ($P = 0.035$) of age at diagnosis 25–44 years. For rs1544410, there showed a significantly different distribution among the different genotypes ($P = 0.011$) of age at diagnosis 65–84 years. More patients carrying the genotype GG or genotype AA had an age of disease onset of

Table 3 Association between the polymorphisms of rs1156820 G/A and rs154410 G/A characteristics among gout patients

dbSNP ID (allele 1/allele 2)*	(1) 1/1* (n)	(2) 1/2* (n)	(3) 2/2* (n)	(1) versus (2) P value	(1) versus (3) P value	(2) versus (3) P value	(1) versus (2 + 3) P value	(1 + 2) versus (3) P value
rs154410 (G/A)	426	58	20					
Demographic characteristics (mean ± SD)								
Age (years)	51.73 ± 13.02	54.57 ± 12.96	56.50 ± 9.11	0.095	0.117	0.564	0.036	0.133
Age at diagnosis (years)	45.88 ± 12.71	48.81 ± 14.58	47.58 ± 9.05	0.325	0.394	0.963	0.102	0.541
Disease duration (years)	10.92 ± 12.22	9.88 ± 8.31	12.20 ± 12.27	0.717	0.529	0.450	0.758	0.604
BMI (kg/m ²)	27.00 ± 3.52	27.12 ± 3.01	27.79 ± 2.58	0.606	0.799	0.462	0.497	0.333
WHR	0.93 ± 0.05	0.92 ± 0.05	0.91 ± 0.06	0.323	0.236	0.776	0.140	0.356
Serum Biochemistry (mean ± SD)								
Systolic pressure (mmHg)	136.23 ± 17.79	144.02 ± 21.86	132.68 ± 19.36	0.007	0.003	0.020	0.032	0.300
Diastolic pressure (mmHg)	89.04 ± 11.37	94.37 ± 15.55	88.32 ± 12.57	0.055	0.993	0.261	0.038	0.626
Blood Glucose (mmol/L)	6.17 ± 1.79	6.13 ± 1.22	6.41 ± 1.30	0.803	0.866	0.520	0.874	0.522
Uric acid (μmol/L)	473.40 ± 115.64	467.17 ± 137.05	466.57 ± 102.55	0.908	0.706	0.984	0.660	0.821
Triglycerides (mmol/L)	2.48 ± 1.92	2.37 ± 1.94	3.31 ± 2.46	0.154	0.691	0.064	0.583	0.058
Total cholesterol (mmol/L)	5.38 ± 1.21	5.33 ± 1.08	5.39 ± 1.19	0.230	0.761	0.104	0.589	0.091
Urea nitrogen (mmol/L)	5.83 ± 2.75	5.69 ± 2.65	5.85 ± 1.55	0.937	0.721	0.830	0.771	0.957
Creatinine (μmol/L)	89.99 ± 26.04	90.21 ± 28.38	87.18 ± 19.21	0.896	0.952	0.660	0.870	0.642
Clinical characteristics								
Age at diagnosis (years)								
<25	10/426	1/58	1/20	0.705	0.765 1.370 (0.172–10.904)	0.455 0.457 (0.056–3.754)	0.908 0.913 (0.196–4.251)	0.433 0.442 (0.054–3.601)
25–44	192/426	25/58	7/20	0.595	0.777	0.376	0.509	0.386
45–64	178/426	32/58	8/20	0.891	1.083 (0.623–1.884)	1.524 (0.596–3.895)	1.179 (0.723–1.925)	1.509 (0.592–3.849)
65–84	46/426	0/58	4/20	0.011	0.660 0.883 (0.509–1.534)	0.874 1.077 (0.431–2.688)	0.766 0.929 (0.571–1.512)	0.849 1.093 (0.439–2.722)
Tophi	99/426	15/58	7/20	0.456	0.009 0.892 (0.863–0.922)	0.263 0.484 (0.155–1.510)	0.124 2.239 (0.782–6.410)	0.126 0.420 (0.135–1.310)
Past history					0.659 0.868 (0.463–1.628)	0.434 0.648 (0.218–1.448)	0.345 0.771 (0.448–1.325)	0.240 0.572 (0.223–1.469)
Hypertension	137/426	18/58	7/20	0.948	0.863	0.791	0.985	0.780
Diabetes	38/426	9/58	2/20	0.282	1.053 (0.583–1.905)	0.880 (0.344–2.256)	1.005 (0.599–1.686)	0.875 (0.342–2.236)
					0.111 0.533 (0.243–1.169)	0.869 0.881 (0.197–3.944)	0.156 0.597 (0.291–1.225)	0.966 0.968 (0.218–4.302)

Table 3 continued

dbSNP ID (allele 1/allele 2)*	(1) 1/1* (n)	(2) 1/2* (n)	(3) 2/2* (n)	(1) versus (2) P value OR (95 % CI)	(1) versus (3) P value OR (95 % CI)	(2) versus (3) P value OR (95 % CI)	(1) versus (2 + 3) P value OR (95 % CI)	(1 + 2) versus (3) P value OR (95 % CI)
Obesity& rs11568820(G/A)	66/426 132	7/58 277	4/20 95	0.494 1.336 (0.581–3.071)	0.588 0.733 (0.238–2.263)	0.380 0.549 (0.142–2.119)	0.754 1.117 (0.560–2.225)	0.549 0.710 (0.231–2.185)
Demographic characteristics (mean ± SD)								
Age (years)	53.22 ± 13.79	51.60 ± 11.99	52.68 ± 14.21	0.465	0.989	0.888	0.304	0.711
Age at diagnosis (years)	46.19 ± 13.60	46.09 ± 12.31	47.27 ± 13.35	0.755	0.547	0.463	0.884	0.455
Disease duration (years)	9.9 ± 11.08	11.97 ± 12.87	8.54 ± 10.38	0.039	0.733	0.034	0.322	0.049
BMI (kg/m ²)	27.08 ± 3.13	27.03 ± 3.64	27.12 ± 3.29	0.973	0.946	0.831	0.921	0.859
WHR	0.92 ± 0.81	0.93 ± 0.5	0.92 ± 0.41	0.683	0.714	0.716	0.427	0.912
Serum biochemistry (mean ± SD)								
Systolic pressure (mmHg)	138.97 ± 18.75	137.12 ± 17.78	134.61 ± 19.91	0.236	0.090	0.272	0.195	0.343
Diastolic pressure (mmHg)	90.05 ± 12.25	90.13 ± 11.42	88.14 ± 13.49	0.386	0.253	0.181	0.738	0.168
Blood Glucose (mmol/L)	6.04 ± 1.55	6.31 ± 1.98	5.94 ± 0.88	0.130	0.679	0.080	0.315	0.161
Uric acid (μmol/L)	468.24 ± 121.13	466.78 ± 108.47	497.54 ± 134.07	0.087	0.070	0.032	0.774	0.038
Triglycerides (mmol/L)	2.39 ± 1.79	2.49 ± 1.88	2.64 ± 2.27	0.651	0.355	0.520	0.515	0.386
Total cholesterol (mmol/L)	5.41 ± 1.26	5.42 ± 1.15	5.28 ± 1.20	0.612	0.441	0.328	0.874	0.325
Urea nitrogen (mmol/L)	5.83 ± 3.17	5.67 ± 2.4	6.38 ± 2.89	0.106	0.146	0.034	0.987	0.041
Creatinine (μmol/L)	96.47 ± 21.30	85.69 ± 24.29	87.99 ± 33.35	0.001	0.121	0.913	0.032	0.770
Clinical characteristics								
Age at diagnosis (years)								
<25	3/132	6/277	3/95	0.857	0.682	0.587	0.924	0.581
25–44	64/132	127/277	33/95	0.094	0.039	0.059	0.277	0.035
45–64	51/132	117/277	49/95	0.139	1.768 (1.027–3.044)	1.591 (0.980–2.581)	1.247 (0.837–1.857)	1.646 (1.034–2.620)
65–84	14/132	27/277	9/95	0.951	0.053	0.114	0.233	0.063
Tophi	30/132	68/277	23/95	0.921	0.591 (0.347–1.008)	0.686 (0.430–1.096)	0.781 (0.521–1.172)	0.654 (0.418–1.024)
Past history					0.780	0.938	0.759	0.871
Hypertension	43/132	95/277	24/95	0.264	1.134 (0.469–2.740)	1.032 (0.467–2.281)	1.107 (0.577–2.125)	1.065 (0.499–2.273)
					0.794	0.947	0.688	0.959
					0.921 (0.495–1.714)	1.019 (0.592–1.754)	0.908 (0.567–1.454)	0.986 (0.586–1.662)
					0.926	0.103	0.901	0.111
					1.028 (0.576–1.836)	1.544 (0.913–2.611)	1.027 (0.672–1.570)	1.506 (0.908–2.499)

Table 3 continued

dbSNP ID	(1) 1/1*	(2) 1/2*	(3) 2/2*	(1) versus (2) versus (3)	(1) versus (2)	(1) versus (3)	(2) versus (3)	(1) versus (2 + 3)	(1 + 2) versus (3)
(allele 1/allele 2)*	(n)	(n)	(n)	P value	OR (95 % CI)	P value	OR (95 % CI)	P value	P value
Diabetes	10/132	31/277	8/95	0.459	0.255 0.650 (0.309–1.370)	0.816 0.891 (0.338–2.350)	0.447 1.370 (0.607–3.095)	0.333 0.700 (0.339–1.445)	0.635 1.212 (0.548–2.677)
Obesity&	21/132	38/277	18/95	0.407	0.365 1.308 (0.731–2.339)	0.741 1.124 (0.561–2.255)	0.219 1.470 (0.793–2.724)	0.568 1.173 (0.677–2.032)	0.315 0.742 (0.414–1.330)

The major allele was referred to as allele 1 and the minor allele as allele 2

Hypertension was defined as systolic blood pressure > 140 mmHg or diastolic blood pressure > 90 mmHg or receiving anti-hypertensive drug treatment in a patient with a history of hypertension. Diabetes was defined on the basis of fasting blood glucose > 7.0 mmol/L (126 mg/dL) or non-fasting blood glucose > 11.1 mmol/L (200 mg/dL) and/or treatment of diabetes. Obesity is defined by the World Health Organization (WHO) as a BMI > 30 kg/m² (36). Statistically significant *P* values are shown in bold

65–84 years compared to those carrying genotypes AG ($P = 0.009$; $P = 0.003$). However, the analysis indicated no significant differences in other stratifications of the age of disease onset and phenotypic disease characteristics such as the duration of gout history, WHR, tophi, or past history.

Discussion

As we know, gout is a kind of disease which belongs to both inflammatory disease and metabolic disease. On the one hand, it is caused by MSU-induced inflammation in the joints and periarticular tissues. The mononuclear phagocytes phagocytize MSU and calcium pyrophosphate dihydrate crystals, and then directly activate the inflammasome through NALP3 [16] in macrophages to release active IL-1 β [17]. IL-1 β activates the production of pro-inflammatory cytokines as well as other inflammatory mediators such as tumor necrosis factor TNF- α , IL-12, IL-6 and IL-8 to lead to a series of reaction [18–23]. On the other hand, gout belongs to a kind of metabolic syndrome, which is a cluster of interrelated conditions characterized by dyslipidemia, hyperglycemia, high blood pressure and abdominal obesity. Metabolic syndrome and its components are associated with serum uric acid levels [24–28], which means the uric acid and metabolic syndrome have some certain close correlation. Hyperuricemia are commonly correlated with glucose intolerance, hypertension and dyslipidemia [3, 24, 29].

Vitamin D is a pleiotropic secosteroid hormone which plays an important role in many systems in human body through its active form 1, 25 (OH) 2 D3. Recently, many researchers have focused on the importance of its non-classical actions in modulating the innate and adaptive immune systems and regulation of cell proliferation, besides its classical function of adjusting calcium uptake and bone metabolism [30]. Studies have shown that the deficiency in vitamin D would cause a series of response, such as the absence of its effect on inhibiting some interleukins like IL-10 from releasing and increase of the expression of IL-12, IL-6 and TNF- α , thus to induce inflammation and decrease the expression of VDR, of which is the highly specific receptor, and prohibition that could be responsible for inhibiting inflammation [31]. So it is much easier to understand that patients with lower serum vitamin D get diseases like many autoimmune diseases and cancer more easily. What is more, vitamin supplements reduced the total kidney mRNA expression of some interleukins which may play important roles in the process of gout such as IL-6, monocyte chemoattractant protein (MCP)-1, and IL-18 [32]. As the specific receptor of vitamin D, VDR combines with vitamin D to form a kind of compound and regulates the transcription and expression of target genes to realize its biological function. Furthermore, it has been reported

that there was a significant decrease of 25-OH levels in the carrier of some certain variant alleles of the polymorphisms of *VDR* compared to the non-carriers [33]. So, we suppose that the polymorphism of *VDR* probably have some association with gout.

Previous GWAS study for the concentration of uric acid has demonstrated moderate associations between several signals and gout [34], but to our best knowledge, no published data have been found about the relationship between *VDR* and gout in a Chinese Han population. Allelic variants on the promoter, coding and 3' UTR region in *VDR* might have effect on its functions [35]. In other vitamin D-related diseases such as osteoporosis and diabetes mellitus, the genotype of *VDR* at the BsmI (rs1544410) restriction site was associated with disease prevalence. In our study, we chose three functional SNPs including rs2228570, rs1544410 and rs11568820 in *VDR* to examine our hypothesis.

In our present study, we chose 504 male patients with gout and 523 ethnically matched gout-free controls to investigate whether the functional *VDR* polymorphisms are associated with susceptibility to gout in Chinese Han male population. Our results showed a significant difference in statistics between rs11568820, rs1544410 and gout, while there was no significant association between gout and rs2228570. We found that the frequencies of the A allele of rs11568820 and A allele of rs1544410 were both higher in gout patients than in controls. Patients with gout had a significantly higher frequency of the AG and GG genotypes than AA in rs11568820 ($P = 0.011$) and rs1544410 ($P = 0.003$). Previous studies have demonstrated that the polymorphisms of *VDR* were associated with many diseases. Bai et al. [36] found that individuals with the GG genotype of rs11568820 were associated with a significant decrease in colorectal cancer risk compared with patients carrying the AA genotype. Gu and his colleague reported that the polymorphism of rs11568820 was significantly associated with BMI and fat mass for the obesity phenotypes [12] ($P = 0.004$). Meanwhile, patients with AA genotype of *VDR* rs1544410 were more likely to get primary hyperparathyroidism than GG genotype according to Carling et al. [13].

However, several limitations in the current study should be considered, such as the small sample size, the single limited area of the population. Additional research is needed to further reevaluate possible relationships between multiple vitamin D pathway genes and the progress of gout in a larger size sample. Overall, these results provide evidence that the polymorphisms of *VDR* might be associated with the risk of gout in Chinese Han male population.

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Conflict of interest None.

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