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Association of *vitamin D receptor* polymorphisms with metabolic syndrome-related components: A cross-sectional study

Ting Jin[1] | Weina Lu[1] | Xiaoqin Gong[2] | Jiaqiang Zhou[1] | Fang Wu[1]

[1]Department of Endocrinology, Zhejiang University School of Medicine Sir Run Run Shaw Hospital, Hangzhou, China

[2]Department of Gynecology, Pujiang People Hospital, Zhejiang, China

Correspondence
Fang Wu, Department of Endocrinology, Zhejiang University School of Medicine Sir Run Run Shaw Hospital, 3 East Qing Chun Road, Hangzhou 310000, Zhejiang, China.
Email: zw5100637@zju.edu.cn

Abstract

Background: The association between vitamin D receptor (*VDR*) polymorphisms and metabolic syndrome (MS) has been demonstrated by epidemiological studies while their correlation remain controversial. The aim of this study is to investigate the association of *VDR* gene polymorphisms with MS and MS-related components in the two communities of Hangzhou.

Methods: A total of 394 subjects were enrolled in the cross-sectional study. Four *VDR* gene polymorphisms (*Apa*I, *Bsm*I, *Fok*I, and *Taq*I) were selected based on human genome sequence databases and genotyped using the MassARRAY Analyzer Compact.

Results: In lipid profile, the TT genotype of *Apa*I had a significantly lower risk of hypertriglyceridemia compared with the GG+GT genotypes (recessive model: OR = 0.141; 95% CI = 0.041–0.486; $p < 0.01$) and the GG genotype (codominant model: OR = 0.155; 95% CI = 0.044–0.545; $p < 0.01$). The levels of triglyceride (TG) in the TT genotype of *Apa*I were lower than the GG+GT genotypes (1.29 ± 0.63 vs. 1.78 ± 1.59 mmol/L, $p < 0.01$). Furthermore, the AA+GA carriers of *Bsm*I had lower levels of high-density lipoprotein cholesterol (HDL-C) than the GG carriers (1.28 ± 0.29 vs. 1.42 ± 0.34 mmol/L, $p < 0.05$). The CC+TC carriers of *Taq*I also suffered from lower HDL-C compared with the TT carriers (1.27 ± 0.29 vs. 1.42 ± 0.34 mmol/L, $p < 0.01$). For arterial blood pressure, the CC carriers had lower systolic blood pressure (SBP) than the TT+TC carriers ($p < 0.01$) and the TT carriers of *Fok*I ($p < 0.05$). However, the *Fok*I polymorphisms were not associated with SBP and the mean blood pressure of both groups laid within the normal range.

Conclusions: In our study, *VDR* polymorphisms show no association with the MS risk. The present results suggest that the *VDR Apa*I polymorphism is associated with hypertriglyceridemia and predisposed to developing MS, while the variants of *Bsm*I and *Taq*I seem to affect HDL-C. Nevertheless, the effect of *Fok*I variants with SBP is ambiguous.

KEYWORDS
blood pressure, lipid, metabolic syndrome, vitamin D receptor polymorphisms

1 | INTRODUCTION

Vitamin D deficiency is a global health problem, with reported incidence of 36% in the United State of America, 61% in Canada, 92% in Northern Europe, 60% in North Africa, 45%–98% in Asia, and 31% in Australia.[1] Vitamin D is traditionally known for its skeletal effects in calcium and phosphorus homeostasis,[2,3] and it appears to have extra-skeletal influence. Low level of vitamin D has been demonstrated to be associated with several non-skeletal disorders including cardiovascular diseases, type 1 and type 2 diabetes, autoimmune diseases, and cancer.[4,5] Currently, the involvement of vitamin D in metabolic syndrome (MS) has attracted great interest. MS comprises a cluster of metabolic components such as obesity, hyperglycemia, hypertension, and hyperlipidemia, which is associated with high risk of cardiovascular disease.[6] Accumulating studies have consistently shown that low vitamin D status is related to obesity in both pediatric and adult populations.[7-11] Given the increasing prevalence of obesity in children, there also has been concern for high occurrence of dyslipidemia, hypertension, and type 2 diabetes.[12] Additionally, the biological influences of vitamin D deficiency on insulin secretion or insulin resistance,[13,14] diabetes,[15-18] hypertension,[19,20] and dyslipidemia[21] have also been reported.

Vitamin D receptor (VDR), which is activated by vitamin D, is expressed in multiple cells. The activated VDR regulates 3% of the human genome, suggesting the pleiotropic functions of vitamin D.[22] Therefore, *VDR* polymorphisms are proposed as the potential genetic contributors to metabolic disorders. Many studies have been made to investigate the role of *VDR* polymorphisms in the pathogenesis of MS. However, the data so far were inconclusive. On one hand, *VDR* genetic variants have been reported to be associated with MS and its components including anthropometric and biochemical parameters in different ethnic populations,[23-29] while on the other hand, some studies have considered that there is no association between *VDR* polymorphisms and MS. In a study on healthy postmenopausal women in Poland, apart from an unfavorable lipid profile, the *Bsm*I polymorphism of *VDR* gene was not associated with the features of MS.[30] Vimaleswaran et al.[31] found no relationship between *VDR* polymorphisms and metabolic components on British adults. In addition, several studies addressed no association of the risk of MS with *VDR* gene polymorphisms in 2013 (*Bsm*I, *Fok*I),[23] 2016 (*Apa*I, *Bsm*I, *Fok*I, *Taq*I),[32] and 2017 (BsmI, *Fok*I, *Taq*I).[26] For Chinese population, only two studies investigated the role of *VDR* gene polymorphisms in MS. In one study, Zhao et al.[33] revealed that there was an association between *Bsm*I *VDR* gene polymorphism and MS in North China. And the other study, which was performed in the rural population of Henan province in North China, showed that the *VDR* gene (rs2228570 *Fok*I, rs3847987, rs2189480, rs2239179) variants were associated with MS.[34]

In this study, we mainly investigate the association of *VDR* gene polymorphisms (*Apa*I, *Bsm*I, *Fok*I, and *Taq*I) with MS and MS-related components in a southern city of China.

2 | MATERIALS AND METHODS

2.1 | Study subjects

A cross-sectional study was performed from April 2010 to February 2011 in the Caihe and Gongshu communities of Hangzhou, Zhejiang Province, China. A total of 394 subjects were recruited, including 56 subjects with MS and 338 controls without MS. The diagnosis of MS was accordance with the criteria by the International Diabetes Federation,[6] which requires central obesity (waist circumference ≥90 cm in men and ≥80 cm in women) plus any two of the following: high blood pressure [systolic blood pressure (SBP) ≥ 130 mmHg or diastolic blood pressure (DBP) ≥85 mmHg], hyperglycemia (fasting blood glucose ≥ 5.6 mmol/L), hypertriglyceridemia [triglyceride (TG) ≥ 1.7 mmol/L (150 mg/dl)], and low high-density lipoprotein cholesterol (HDL-C) [<1.03 mmol/L (40 mg/dl) in men and <1.29 mmol/L (50 mg/dl) in women]. The unmatched subjects were placed in the control group. Exclusion criteria included: use of vitamin D, pregnant or breastfeeding women, chronic kidney diseases, and other chronic illness. None of the subjects used the hypotensive drugs, lipid-lowering medications, oral hypoglycemic agents, and/or insulin. A written informed consent was obtained from each participant. The study was approved by the ethic committee of Sir Run Run Shaw Hospital.

2.2 | Anthropometric and biochemical measurements

All participants were subjected to anthropometric measurements including body mass index (BMI), waist circumference (WC), SBP, and DBP. All measurements were performed by well-trained researchers. BMI was calculated by dividing the body weight in kilogram by the stand height in meter squared. WC was measured with an inelastic tape placed at the midpoint between the lower rib margin and the iliac crest, while the subject was standing up. Blood pressure was obtained using an automatic sphygmomanometer (Omron Health Care). On the day of the questionnaire, the first measurement was performed after at least a rest period of 5 min, and the second was performed 30 min after the first measurement. The third measurement was performed on the next day. Three measurements were taken, and the average of the measurements was used for analysis.

Venous blood samples were collected at morning after an overnight fast. Each blood specimen was divided into two sections, one section of 8 ml was transferred into the coagulation-promoting tube, and the other 8 ml was transferred into the ethylene diamine tetraacetic acid (EDTA)-anticoagulated tubes. Then, the blood samples were stored at −80°C after centrifuge until used. Blood tests with biochemistry were conducted from the coagulation-promoting tubes. Serum fasting blood glucose (FPG), TG, total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), and HDL-C were determined with the automated biochemistry analyzer (Aeroset). The detection procedures and

reaction criteria were set and carried out strictly according to the instruction of manufacturer.

2.3 | Genetic analyses

Genomic DNA was extracted from EDTA-anticoagulated whole blood with QIAGEN DNA Mini and Blood Mini Kit (QIAGEN). Four *VDR* single-nucleotide polymorphisms (SNPs), rs7975232 (*Apa*I), rs1544410 (*Bsm*I), rs2228570 (*Fok*I), and rs731236 (*Taq*I), were identified based on human genome sequence databases. The genotypes of the four *VDR* SNPs were determined by polymerase chain reaction (PCR) using iPlex assay (Sequenom). The PCR reactions (5 µl) were performed in standard 384-well plates using 3 µl of reaction mixture (0.625 µl buffer, 0.325 µl MgCl$_2$, 0.1 µl dNTP, 0.12 µl PCR primer, 0.1 µl Taqpolymerase, and 1.73 µl ddH$_2$O) and 2 µl DNA. The cycling was carried out for 2 min at 95°C, followed by 45 cycles of 30 s at 95°C, 30 s at 56°C, and 60 s at 72°C, and then 5 min at 72°C. Shrimp Alkaline Phosphatase (SAP) mixture (2 µl), containing 0.17 µl SAP buffer, 0.3 µl SAP enzyme, and 1.53 µl ddH$_2$O, was added to the completed reactions and incubated for 40 min at 37°C, followed by inactivation for 5 min at 85°C. Final extension was continued with 2 µl mixture (0.2 µl iPlex buffer, 0.2 µl iPlex termination, 0.94 µl primer, 0.041 µl iPlex enzyme, and 0.619 µl ddH$_2$O). The cycling was performed for 30 s at 94°C, followed by 5 cycles of 5 s at 94°C, 5 s at 52°C, and 5 s at 80°C. The condition consisted of 40 cycles of denaturation at 94°C and was completed by 3 min at 72°C. The reaction products were added with 6 mg Clean Resin for purify. The products were spotted on the Spectrochip (Sequenom), and the data were analyzed using the MassARRAY Analyzer Compact (Sequenom). A reagent control (without template DNA) was included in each PCR, and 5% of the samples were randomly selected for repeated detection to avoid genotyping errors.

2.4 | Statistical analysis

All statistical analyses were implemented by using SPSS, and all data were presented as mean ± standard deviation. The Hardy-Weinberg equilibrium using the chi-square test was performed for each SNP before analysis. The difference between the two groups was analyzed by the Student *t*-test for numeric data. For multiple comparisons among the groups, the one-way ANOVA was conducted. The associations of the SNPs with MS and MS-related components were evaluated using the binary logistic regression, after adjustment for age, sex, and BMI. A P-value <0.05 was considered statistically significant.

3 | RESULTS

In this study, the mean age of the controls was 55.70 ± 6.55 years and the mean age of the MS was 57.50 ± 6.41 years. As shown in

TABLE 1 Baseline characteristics of subjects with controls and MS

Characteristics	Controls	MS	*p*-Value
Subjects (*n*)	338	56	-
Age (years)	55.70 ± 6.55	57.50 ± 6.41	0.056
BMI (kg/m^2)	22.89 ± 2.48	26.55 ± 2.60	<0.001
WC (cm)	76.68 ± 7.98	88.76 ± 6.52	<0.001
SBP (mmHg)	117.93 ± 14.58	130.80 ± 15.08	<0.001
DBP (mmHg)	78.24 ± 8.73	84.80 ± 10.02	<0.001
FPG (mmol/L)	5.00 ± 1.08	5.84 ± 1.93	0.002
TG (mmol/L)	1.61 ± 1.51	2.34 ± 1.43	<0.001
TC (mmol/L)	5.56 ± 1.21	5.63 ± 1.00	0.666
LDL-C (mmol/L)	2.41 ± 0.66	2.45 ± 0.60	0.709
HDL-C (mmol/L)	1.44 ± 0.34	1.22 ± 0.28	<0.001

Note: Data were presented as mean ± standard deviation. *p*-value obtained by Student *t*-test. A *p*-value <0.05 was considered statistically significant.

Abbreviation: BMI, body mass index; DBP, diastolic blood pressure; FPG, fasting blood glucose; HDL-C, high-density lipoprotein; LDL-C, low-density lipoprotein; MS, metabolic syndrome; SBP, systolic blood pressure; TC, total cholesterol; TG, triglyceride; WC, waist circumference.

Table 1, there was significant difference in clinical and laboratory measurements between the control and MS groups. The results found that there were significantly higher levels of BMI, WC, SBP, DBP, FPG, TG, and lower HDL-C level in the subjects with MS as compared with those in the control group. However, the levels of TC and LDL-C did not differ between the two groups.

Genotyping for all the SNPs in *VDR* gene was performed in both the MS group and the control group. Four *VDR* polymorphisms of all subjects, control, and MS groups are shown in Table 2. It was observed that there was no significant difference in the genotype distribution within the groups. The genotype distributions of four SNPs in *VDR* gene were consistent with Hardy-Weinberg equilibrium (*p* > 0.05).

After adjusting for age, sex, and BMI, the associations between *Apa*I, *Bsm*I, *Fok*I, *and Taq*I with MS and MS-related components are presented in Table 3. All four *VDR* SNPs were not related to MS risk (*p* > 0.05). However, in the case of *Apa*I TT genotype, the two models in comparison with hypertriglyceridemia were statistically significant. The first one was recessive model with the GG+GT genotypes (OR = 0.141; 95% CI = 0.041–0.486; *p* = 0.002), and the second one was codominant model with the GG genotype (OR = 0.155; 95% CI = 0.044–0.545; *p* = 0.004).

We further explored the differences in the levels of MS-related components by genotypes of *VDR* (Table 4). In the lipid profile, the levels of TG in TT genotype of *Apa*I were lower than GG+GT genotypes (1.29 ± 0.63 vs. 1.78 ± 1.59 mmol/L, *p* = 0.001). The HDL-C levels were lower in the subjects with AA+GA than GG genotype of *Bsm*I (1.28 ± 0.29 vs. 1.42 ± 0.34 mmol/L, *p* = 0.016). The subjects with CC+TC genotype of *Taq*I polymorphism had significant lower levels of HDL-C compared with TT genotype (1.27 ± 0.29

TABLE 2 Allele and genotype frequencies of *VDR* polymorphisms in the study subjects

Genotype/allele	All subjects (n = 394)	Controls (n = 338)	MS (n = 56)
*Taq*I allele			
T	729 (94.68)	622 (94.53%)	107 (95.54%)
C	41 (5.32%)	36 (5.47%)	5 (4.46%)
*Taq*I genotype			
TT	346 (89.87%)	295 (89.67%)	51 (91.07%)
TC	37 (9.61%)	32 (9.73%)	5 (8.93%)
CC	2 (0.52%)	2 (0.61%)	0
*Bsm*I allele			
G	735 (94.96%)	628 (94.86%)	107 (95.54%)
A	39 (5.04%)	34 (5.14%)	5 (4.46%)
*Bsm*I genotype			
GG	350 (90.44%)	299 (90.33%)	51 (91.07%)
GA	35 (9.04%)	30 (9.06%)	5 (8.93%)
AA	2 (0.52%)	2 (0.61%)	0
*Apa*I allele			
G	548 (76.54%)	467 (71.19%)	81 (73.64%)
T	218 (28.46%)	189 (28.81%)	29 (26.36%)
*Apa*I genotype			
GG	199 (51.96%)	172 (52.44%)	27 (49.09%)
GT	150 (39.16%)	123 (37.50%)	27 (49.09%)
TT	34 (8.88%)	33 (10.06%)	1 (1.82%)
*Fok*I allele			
T	380 (48.97%)	322 (48.49%)	58 (51.79%)
C	396 (51.03%)	342 (51.51%)	54 (48.21%)
*Fok*I genotype			
TT	98 (25.26%)	80 (24.10%)	18 (32.14%)
TC	184 (57.42%)	162 (48.80%)	22 (39.29%)
CC	106 (27.32%)	90 (27.10%)	16 (28.57%)

Abbreviation: MS, metabolic syndrome; VDR, vitamin D receptor.

vs. 1.42 ± 0.34 mmol/L, p = 0.008). In arterial blood pressure measurement, the *Fok*I CC genotype had lower SBP than the TT+TC genotypes (116.45 ± 15.74 vs. 121.00 ± 14.99 mmHg, p = 0.009) and the TT genotype (116.45 ± 15.74 vs. 121.60 ± 16.37 mmHg, p = 0.016), but the mean values of SBP were both within the normal range. There were no statistically differences in other components.

4 | DISCUSSION

Our study revealed that the *VDR* gene polymorphisms (*Apa*I, *Bsm*I, *Fok*I, and *Taq*I) were not associated with MS. In contrast, previous studies in northern cities of China showed the associations of *VDR* gene polymorphisms with MS.[33,34] These inconsistent results with our study in southern city may be due to the heterogeneity of

sample, and diagnostic criteria of MS. In this study, we found that *VDR* gene polymorphisms may affect the MS-related components, especially the lipid profile.

Dyslipidemia is the component of MS, which includes hypertriglyceridemia and low HDL-C. Vitamin D has been proposed to have effects on the lipid metabolism. There were numerous epidemiologic studies to explore the association of vitamin D status with dyslipidemia. A systemic review, including 22 cross-section studies, found that vitamin D status was positively related to serum HDL-C and negatively to TG.[21] The adipose tissue, which expresses VDR and enzymes involving in vitamin D metabolism and related-signaling, is the major storage site for vitamin D.[35] The multiple functions of vitamin D are mediated by VDR, which heterodimerizes with the retinoid X receptor (RXR). The vitamin D-VDR interaction results in alternating gene expression and protein synthesis.[36] In addition, *VDR* gene expression is up-regulated by vitamin D in 3T3-L1 preadipocytes.[37] These results suggest that the influence of vitamin D on the lipid metabolism is VDR dependent. Vitamin D and VDR are supposed to be implicated in the modulation of adipogenesis. The studies using 3T3-L1 mouse preadipocytes suggested that vitamin D could inhibit adipogenesis.[38-40] Additionally, further researches indicated that the liganded VDR blocked adipogenensis through downregulating the expression of adipogenic transcription factors.[38,39] Nevertheless, a role for unliganded VDR in either promoting or inhibiting adipogenesis has been reported. In 3T3-L1 preadipocytes, adenovirus-mediated overexpression of VDR suppressed the adipogenic program,[38] while knockdown of VDR using small interfering RNA reduced lipid accumulation in the late phase of adipogenesis.[39] Interestingly, the effects on human adipocytes were not consistent where vitamin D promoted adipocyte differentiation.[41-43]

The perturbation of lipid profile has been described in VDR knockout animals. Wong et al.[44] showed that VDR-null mutant mice exhibited the lower levels of serum triglycerides and cholesterol levels than the wild type, due to increased rate of fatty acid β-oxidation. Further study demonstrated that the transgenic mice overexpressing VDR showed an increase in the levels of serum cholesterol, which was correlated with reduced fatty acid β-oxidation and lipolysis in the adipose tissue.[45] Besides, high expression of VDR suppressed the expressions of genes that regulated thermogenesis and lipolysis in adipose tissue.[46]

Our study found the associations between *Apa*I, *Bsm*I, and *Taq*I variants with lipid profile. Logistic regression analysis showed a significant association between *Apa*I TT genotype with hypertriglyceridemia. In Russian subjects with MS, T allele carriers of *VDR Apa*I had higher TC and LDL-C levels compared with GG carriers.[28] Moreover, AA+GA carriers of *Bsm*I and CC+TC carriers of *Taq*I showed lower HDL-C levels. Current data demonstrated that *VDR Bsm*I gene polymorphism might be closely associated with dyslipidemia. A previous study revealed the similar finding that AA genotype and A allele of *Bsm*I were associated with lower HDL-C and obesity, respectively.[25] Furthermore, AA carriers of *Bsm*I were related to high LDL-C levels in Polish postmenopausal women,[30]

TABLE 3 Association of VDR gene polymorphisms with MS and MS-related components

SNP	MS OR (95% CI)	P	Central obesity OR (95% CI)	P	Hypertension OR (95% CI)	P	Hyperglycemia OR (95% CI)	P	Hypertriglyceridemia OR (95% CI)	P	Low HDL-C OR (95% CI)	P
TaqI												
Allele												
T												
C	0.732 (0.237–2.262)	0.588	2.205 (0.779–6.236)	0.136	0.927 (0.435–1.976)	0.845	1.106 (0.471–2.597)	0.817	0.774 (0.382–1.570)	0.478	1.849 (0.956–3.576)	0.068
Dominant												
TT												
CC+TC	0.941 (0.298–2.977)	0.918	2.417 (0.800–7.300)	0.118	1.099 (0.502–2.408)	0.813	0.981 (0.384–2.502)	0.967	0.862 (0.413–1.799)	0.692	1.734 (0.858–3.503)	0.125
Recessive												
TT+TC												
CC	-	-	0.841 (0.000–1690.317)	0.964	-	-	4.322 (0.251–74.391)	0.313	-	-	-	-
Codominant												
TT												
CC	-	-	0.960 (0.035–26.077)	0.981	-	-	0.465 (0.112–1.932)	0.292	-	-	-	-
TC	1.037 (0.826–1.302)	0.754	1.198 (0.958–1.497)	0.113	1.052 (0.898–1.232)	0.532	0.968 (0.791–1.183)	0.748	0.993 (0.856–1.152)	0.926	1.093 (0.946–1.263)	0.229
BsmI												
Allele												
G												
A	0.753 (0.242–2.347)	0.625	1.737 (0.587–5.140)	0.319	0.876 (0.401–1.915)	0.740	1.191 (0.504–2.813)	0.691	0.732 (0.353–1.518)	0.402	1.810 (0.922–3.554)	0.085
Dominant												
GG												
AA+GA	0.979 (0.307–3.118)	0.971	1.842 (0.588–5.770)	0.294	1.044 (0.465–2.343)	0.917	1.064 (0.414–2.731)	0.898	0.817 (0.383–1.740)	0.600	1.681 (0.819–3.451)	0.157

(Continues)

TABLE 3 (Continued)

SNP	MS OR (95% CI)	P	Central obesity OR (95% CI)	P	Hypertension OR (95% CI)	P	Hyperglycemia OR (95% CI)	P	Hypertriglyceridemia OR (95% CI)	P	Low HDL-C OR (95% CI)	P
Recessive												
GG+GA												
AA	-	-	0.840 (0.000–1741.041)	0.964	-	-	4.334 (0.252–74.661)	0.313	-	-	-	-
Codominant												
GG												
AA	-	-	1.008 (0.034–29.732)	0.996	-	-	0.464 (0.112–1.928)	0.291	-	-	-	-
GA	1.079 (0.737–1.580)	0.697	1.232 (0.840–1.808)	0.286	1.072 (0.817–1.408)	0.616	0.973 (0.695–1.363)	0.875	0.972 (0.754–1.254)	0.829	1.145 (0.894–1.466)	0.283
ApaI												
Allele												
G												
T	0.924 (0.542–1.576)	0.772	0.837 (0.468–1.496)	0.547	0.894 (0.612–1.306)	0.563	1.115 (0.731–1.702)	0.612	0.733 (0.514–1.046)	0.087	1.140 (0.804–1.618)	0.462
Dominant												
GG												
TT+GT	1.291 (0.652–2.558)	0.464	0.898 (0.427–1.885)	0.775	1.055 (0.651–1.711)	0.828	1.196 (0.691–2.070)	0.522	0.953 (0.611–1.486)	0.832	1.202 (0.766–1.887)	0.423
Recessive												
GG+GT												
TT	0.135 (0.016–1.171)	0.069	0.515 (0.116–2.280)	0.382	0.446 (0.173–1.150)	0.095	1.008 (0.388–2.613)	0.988	0.141 (0.041–0.486)	0.002[*]	1.106 (0.496–2.465)	0.806
Codominant												
GG												
TT	0.171 (0.019–1.521)	0.113	0.514 (0.112–2.364)	0.393	0.488 (0.184–1.292)	0.149	1.100 (0.409–2.960)	0.850	0.155 (0.044–0.545)	0.004[**]	1.201 (0.524–2.754)	0.665
GT	1.64 (0.810–3.317)	0.169	0.997 (0.459–2.164)	0.993	1.230 (0.741–2.042)	0.424	1.219 (0.684–2.171)	0.501	1.248 (0.786–1.981)	0.347	1.203 (0.749–1.931)	0.445

(Continues)

TABLE 3 (Continued)

SNP	MS OR (95% CI)	P	Central obesity OR (95% CI)	P	Hypertension OR (95% CI)	P	Hyperglycemia OR (95% CI)	P	Hypertriglyceridemia OR (95% CI)	P	Low HDL-C OR (95% CI)	P
FokI												
Allele												
T												
C	0.772 (0.479–1.243)	0.287	1.147 (0.679–1.938)	0.608	0.773 (0.550–1.086)	0.137	1.153 (0.782–1.698)	0.472	0.899 (0.658–1.229)	0.505	1.176 (0.856–1.615)	0.317
Dominant												
TT												
CC+TC	0.640 (0.308–1.329)	0.231	1.499 (0.635–3.539)	0.356	0.798 (0.465–1.370)	0.413	1.348 (0.700–2.595)	0.371	1.023 (0.616–1.698)	0.931	1.296 (0.761–2.205)	0.340
Recessive												
TT+TC												
CC	0.820 (0.378–1.778)	0.616	0.948 (0.406–2.216)	0.902	0.637 (0.361–1.122)	0.118	1.085 (0.588–2.000)	0.794	0.743 (0.447–1.237)	0.253	1.181 (0.719–1.939)	0.511
Codominant												
TT												
CC	0.611 (0.240–1.555)	0.302	1.176 (0.374–3.691)	0.782	0.608 (0.307–1.204)	0.153	1.313 (0.598–2.882)	0.497	0.844 (0.451–1.579)	0.595	1.393 (0.748–2.595)	0.296
TC	0.651 (0.294–1.440)	0.289	1.643 (0.658–4.106)	0.288	0.921 (0.514–1.651)	0.783	1.332 (0.668–2.658)	0.415	1.158 (0.673–1.994)	0.596	1.266 (0.715–2.241)	0.418

Note: P-value obtained by binary logistic regression after adjustment for age, sex, and BMI. A P-value <0.05 was considered statistically significant.

Abbreviation: HDL-C, high-density lipoprotein; MS, metabolic syndrome; SNP, single nucleotide polymorphism; VDR, vitamin D receptor.

*p = 0.002 compared with GG+GT genotypes.; **p = 0.004 compared with GG genotype.

TABLE 4 MS-related components in relation to *VDR* gene polymorphisms

SNP	WC	SBP	DBP	TG	TC	HDL-C	LDL-C	FPG
TaqI								
Dominant								
TT	78.24 ± 8.78	119.78 ± 15.49	79.11 ± 9.26	1.72 ± 1.56	5.58 ± 1.18	1.42 ± 0.34	2.43 ± 0.65	5.11 ± 1.23
CC+TC	79.67 ± 9.56	119.64 ± 14.46	79.95 ± 8.19	1.86 ± 1.31	5.47 ± 1.25	1.27 ± 0.29[*]	2.33 ± 0.70	5.34 ± 1.69
BsmI								
Dominant								
GG	78.23 ± 8.75	119.85 ± 15.38	79.14 ± 9.23	1.72 ± 1.56	5.58 ± 1.17	1.42 ± 0.34	2.43 ± 0.65	5.11 ± 1.22
AA+GA	79.73 ± 9.73	118.86 ± 15.06	79.49 ± 8.31	1.83 ± 1.25	5.49 ± 1.29	1.28 ± 0.29[**]	2.35 ± 0.72	5.36 ± 1.73
ApaI								
Recessive								
GG+GT	78.39 ± 8.77	119.71 ± 15.67	79.28 ± 9.14	1.78 ± 1.59	5.60 ± 1.21	1.41 ± 0.33	2.43 ± 0.67	5.12 ± 1.28
TT	78.27 ± 10.03	120.15 ± 12.56	77.88 ± 9.29	1.29 ± 0.63[***]	5.28 ± 0.84	1.42 ± 0.36	2.28 ± 0.44	5.23 ± 1.31
FokI								
Recessive								
TT+TC	78.19 ± 8.68	121.00 ± 14.99	79.68 ± 9.16	1.79 ± 1.70	5.63 ± 1.25	1.43 ± 0.34	2.44 ± 0.69	5.14 ± 1.29
CC	78.73 ± 9.37	116.45 ± 15.74[****]	77.83 ± 8.92	1.56 ± 0.94	5.43 ± 0.97	1.36 ± 0.31	2.36 ± 0.56	5.10 ± 1.24
Codominant								
TT	78.63 ± 8.63	121.60 ± 16.37	79.49 ± 9.72	1.59 ± 0.86	5.75 ± 1.28	1.44 ± 0.32	2.55 ± 0.68	5.06 ± 0.94
CC	78.73 ± 9.37	116.45 ± 15.74[*****]	77.83 ± 8.92	1.56 ± 0.94	5.43 ± 0.97	1.36 ± 0.31	2.36 ± 0.56	5.10 ± 1.24
TC	77.96 ± 8.72	120.68 ± 14.25	79.78 ± 8.87	1.90 ± 2.00	5.57 ± 1.24	1.42 ± 0.35	2.39 ± 0.69	5.18 ± 1.44

Note: Data were presented as mean ± standard deviation. *P*-value obtained by Student *t*-test or one-way ANOVA. A *P*-value <0.05 was considered statistically significant.

Abbreviation: DBP, diastolic blood pressure; FPG, fasting blood glucose; HDL-C, high-density lipoprotein; LDL-C, low-density lipoprotein; SBP, systolic blood pressure; SNP, single nucleotide polymorphism; TC, total cholesterol; TG, triglyceride; VDR, vitamin D receptor; WC, waist circumference.

[*]$p = 0.008$ compared with TT genotype.; [**]$p = 0.016$ compared with GG genotype.; [***]$p = 0.001$ compared with GG+GT genotypes.; [****]$p = 0.009$ compared with TT+TC genotypes.; [*****]$p = 0.016$ compared with TT genotype.

whereas GA carriers showed higher LDL-C than AA+GG carriers in the United Arab Emirates.[26] Karonova et al.[28] and Sangkaew et al.[29] observed the relationship of *Bsm*I with hypertriglyceridemia. Unlike *VDR Bsm*I and *Apa*I, no association between *Taq*I gene polymorphism and lipid profile was reported in MS subjects. Our results were consistent with some and inconsistent with other previous studies, particularly on the association between *Bsm*I polymorphism and lipid profile.

Genetic polymorphisms have subtle but truly biological effects. Genetic studies of *VDR* polymorphisms in relation to metabolic disorders have received more attention. *Apa*I, *Bsm*I (in the intron between exon 8 and 9), and *Taq*I (in exon 9), which are located at the 3′ untranslated region (UTR) of *VDR* gene, were the most frequently studied.[47] Whitfield et al. showed that *VDR* variants (*Apa*I, *Bsm*I, and *Taq*I) displayed cell-type-specific effects on mRNA stability in vitro,[48] whereas other studies showed differences in VDR mRNA expression.[49-52] The in vivo studies also found that the functional effects of *VDR* polymorphisms have been analyzed in the serum biochemical markers.[53-55] These markers, including bone metabolism markers and parathyroid hormone, are thought to be related to the bone mineral density. Thus, *VDR* variants (*Taq*I, *Bsm*I, and *Apa*I) may mediate a host of downstream effects on *VDR*-responsive genes to influence epidemiological findings. However, several studies were not able to explore the differences in mRNA stability, mRNA expression, and serum markers, even demonstrated an opposite trend.[56] Therefore, controversy still exists regarding the functionality of 3′ UTR polymorphisms (*Apa*I, *Bsm*I, and *Taq*I). The genetic information can partially explain the inconsistent association of 3′ UTR polymorphisms with lipid indexes from epidemiological results.

An increasing number of clinical studies confirmed the association of hypertension with vitamin D deficiency.[19] The hypothesized mechanisms through vitamin D impact blood pressure include renin-angiotensin-aldosterone system (RAAS), calcium homeostasis, vascular endothelia function, and inflammation.[57-59] A study found the association between *VDR Fok*I polymorphism and hypertension risk in Chinese population. In another study on Arabs adult, Hasan et al. found that T allele of *VDR Fok*I had higher SBP than C allele. However, our results did not show this. In our study, the data showed the difference of SBP values among genotypes of *Fok*I, but the mean values were within the normal range.

Several limitations of the current study deserve consideration. Firstly, since our sample size is not large enough, further studies with large sample size are required to validate our results. Secondly, the number of subjects available for minor allele frequency of some SNPs was a little low to calculate a strong power. Thirdly, we did not include all *VDR* polymorphisms to investigate. Thus, we cannot rule out other *VDR* polymorphisms that may associate with MS and MS-related components in this population.

In conclusion, VDR gene polymorphisms (*Apa*I, *Bsm*I, *Fok*I, and *Taq*I) were not associated with MS risk in the subjects from the southern city of China. Yet, *VDR* gene polymorphisms may affect the components of MS.

ORCID

Fang Wu https://orcid.org/0000-0001-9178-133X

REFERENCES

1. Holick MF. The vitamin D deficiency pandemic: approaches for diagnosis, treatment and prevention. *Rev Endocr Metab Disord*. 2017;18(2):153-165.
2. Holick MF, Binkley NC, Bischoff-Ferrari HA, et al. Guidelines for preventing and treating vitamin D deficiency and insufficiency revisited. *J Clin Endocrinol Metab*. 2012;97(4):1153-1158.
3. Holick MF, Binkley NC, Bischoff-Ferrari HA, et al. Evaluation, treatment, and prevention of vitamin D deficiency: an Endocrine Society clinical practice guideline. *J Clin Endocrinol Metab*. 2011;96(7):1911-1930.
4. Marino R, Misra M. Extra-skeletal effects of vitamin D. *Nutrients*. 2019;11(7):1460-1483.
5. Marquina C, Mousa A, Scragg R, et al. Vitamin D and cardiometabolic disorders: a review of current evidence, genetic determinants and pathomechanisms. *Obes Rev*. 2019;20(2):262-277.
6. Alberti KG, Zimmet P, Shaw J. Metabolic syndrome–a new worldwide definition. A Consensus Statement from the International Diabetes Federation. *Diabet Med*. 2006;23(5):469-480.
7. Olson ML, Maalouf NM, Oden JD, et al. Vitamin D deficiency in obese children and its relationship to glucose homeostasis. *J Clin Endocrinol Metab*. 2012;97(1):279-285.
8. Arunabh S, Pollack S, Yeh J, et al. Body fat content and 25-hydroxyvitamin D levels in healthy women. *J Clin Endocrinol Metab*. 2003;88(1):157-161.
9. Looker AC. Body fat and vitamin D status in black versus white women. *J Clin Endocrinol Metab*. 2005;90(2):635-640.
10. Bischof MG, Heinze G, Vierhapper H. Vitamin D status and its relation to age and body mass index. *Horm Res*. 2006;66(5):211-215.
11. Lagunova Z, Porojnicu AC, Lindberg F, et al. The dependency of vitamin D status on body mass index, gender, age and season. *Anticancer Res*. 2009;29(9):3713-3720.
12. Misra M, Pacaud D, Petryk A, et al. Vitamin D deficiency in children and its management: review of current knowledge and recommendations. *Pediatrics*. 2008;122(2):398-417.
13. Lee S, Clark SA, Gill RK, et al. 1,25-Dihydroxyvitamin D3 and pancreatic beta-cell function: vitamin D receptors, gene expression, and insulin secretion. *Endocrinology*. 1994;134(4):1602-1610.
14. Boucher BJ, John WG, Noonan K. Hypovitaminosis D is associated with insulin resistance and beta cell dysfunction. *Am J Clin Nutr*. 2004;80(6):1666.
15. Song Y, Wang L, Pittas AG, et al. Blood 25-hydroxy vitamin D levels and incident type 2 diabetes: a meta-analysis of prospective studies. *Diabetes Care*. 2013;36(5):1422-1428.
16. Mauss D, Jarczok MN, Hoffmann K, et al. Association of vitamin D levels with type 2 diabetes in older working adults. *Int J Med Sci*. 2015;12(5):362-368.

17. Bellou V, Belbasis L, Tzoulaki I, et al. Risk factors for type 2 diabetes mellitus: An exposure-wide umbrella review of meta-analyses. *PLoS ONE*. 2018;13(3):e0194127.

18. Gupta A, Aslam M, Rathi S, et al. Association of vitamin D levels and type 2 diabetes mellitus in Asian Indians is Independent of obesity. *Exp Clin Endocrinol Diabetes*. 2018;126(9):553-558.

19. Burgaz A, Orsini N, Larsson SC, et al. Blood 25-hydroxyvitamin D concentration and hypertension: a meta-analysis. *J Hypertens*. 2011;29(4):636-645.

20. Kunutsor SK, Apekey TA, Steur M. Vitamin D and risk of future hypertension: meta-analysis of 283,537 participants. *Eur J Epidemiol*. 2013;28(3):205-221.

21. Jorde R, Grimnes G. Vitamin D and metabolic health with special reference to the effect of vitamin D on serum lipids. *Prog Lipid Res*. 2011;50(4):303-312.

22. Haussler MR, Jurutka PW, Mizwicki M, et al. Vitamin D receptor (VDR)-mediated actions of 1alpha,25(OH)(2)vitamin D(3): genomic and non-genomic mechanisms. *Best Pract Res Clin Endocrinol Metab*. 2011;25(4):543-559.

23. Schuch NJ, Garcia VC, Vivolo SR, et al. Relationship between Vitamin D Receptor gene polymorphisms and the components of metabolic syndrome. *Nutr J*. 2013;12:96-103.

24. Filus A, Trzmiel A, Kuliczkowska-Plaksej J, et al. Relationship between vitamin D receptor BsmI and FokI polymorphisms and anthropometric and biochemical parameters describing metabolic syndrome. *Aging Male*. 2008;11(3):134-139.

25. Al-Daghri NM, Al-Attas OS, Alkharfy KM, et al. Association of VDR-gene variants with factors related to the metabolic syndrome, type 2 diabetes and vitamin D deficiency. *Gene*. 2014;542(2):129-133.

26. Hasan HA, AbuOdeh RO, Muda W, et al. Association of Vitamin D receptor gene polymorphisms with metabolic syndrome and its components among adult Arabs from the United Arab Emirates. *Diabetes Metab Syndr*. 2017;11(suppl 2):531-537.

27. Beydoun MA, Hossain S, Tajuddin SM, et al. Vitamin D metabolism-related gene haplotypes and their association with metabolic disturbances among African-American urban adults. *Sci Rep*. 2018;8(1):8035-8046.

28. Karonova T, Grineva E, Belyaeva O, et al. Relationship between vitamin D status and vitamin D receptor gene polymorphisms with markers of metabolic syndrome among adults. *Front Endocrinol (Lausanne)*. 2018;9:448.

29. Sangkaew B, Nuinoon M, Jeenduang N. Association of vitamin D receptor gene polymorphisms with serum 25(OH)D levels and metabolic syndrome in Thai population. *Gene*. 2018;659:59-66.

30. Tworowska-Bardzinska U, Lwow F, Kubicka E, et al. The vitamin D receptor gene BsmI polymorphism is not associated with anthropometric and biochemical parameters describing metabolic syndrome in postmenopausal women. *Gynecol Endocrinol*. 2008;24(9):514-518.

31. Vimaleswaran KS, Power C, Hypponen E. Interaction between vitamin D receptor gene polymorphisms and 25-hydroxyvitamin D concentrations on metabolic and cardiovascular disease outcomes. *Diabetes Metab*. 2014;40(5):386-389.

32. Shab-Bidar S, Neyestani TR, Djazayery A. Vitamin D receptor gene polymorphisms, metabolic syndrome, and type 2 diabetes in Iranian subjects: no association with observed SNPs. *Int J Vitam Nutr Res*. 2016;86(1–2):71-80.

33. Zhao Y, Liao S, He J, et al. Association of vitamin D receptor gene polymorphisms with metabolic syndrome: a case-control design of population-based cross-sectional study in North China. *Lipids Health Dis*. 2014;13:129-135.

34. Wang J, Wang Y, Han H, et al. Association of Vitamin D Receptor Gene Polymorphisms with Metabolic Syndrome in Rural Areas of China. *Biomed Environ Sci*. 2019;32(4):304-308.

35. Landrier JF, Karkeni E, Marcotorchino J, et al. Vitamin D modulates adipose tissue biology: possible consequences for obesity? *Proc Nutr Soc*. 2016;75(1):38-46.

36. Walters MR, Nemere I. Receptors for steroid hormones: membrane-associated and nuclear forms. *Cell Mol Life Sci*. 2004;61(18):2309-2321.

37. Kamei Y, Kawada T, Kazuki R, et al. Vitamin D receptor gene expression is up-regulated by 1,25-dihydroxyvitamin D3 in 3T3-L1 preadipocytes. *Biochem Biophys Res Commun*. 1993;193(3):948-955.

38. Blumberg JM, Tzameli I, Astapova I, et al. Complex role of the vitamin D receptor and its ligand in adipogenesis in 3T3-L1 cells. *J Biol Chem*. 2006;281(16):11205-11213.

39. Kong J, Li YC. Molecular mechanism of 1,25-dihydroxyvitamin D3 inhibition of adipogenesis in 3T3-L1 cells. *Am J Physiol Endocrinol Metab*. 2006;290(5):E916-924.

40. Rayalam S, Della-Fera MA, Ambati S, et al. Enhanced effects of 1,25(OH)(2)D(3) plus genistein on adipogenesis and apoptosis in 3T3-L1 adipocytes. *Obesity*. 2008;16(3):539-546.

41. Shi H, Norman AW, Okamura WH, et al. 1alpha,25-Dihydroxyvitamin D3 modulates human adipocyte metabolism via nongenomic action. *FASEB J*. 2001;15(14):2751-2753.

42. Nimitphong H, Holick MF, Fried SK, et al. 25-Hydroxyvitamin D-3 and 1,25-Dihydroxyvitamin D-3 promote the differentiation of human subcutaneous preadipocytes. *PLoS ONE*. 2012;7(12):e52171.

43. Narvaez CJ, Simmons KM, Brunton J, et al. Induction of STEAP4 correlates with 1,25-dihydroxyvitamin D3 stimulation of adipogenesis in mesenchymal progenitor cells derived from human adipose tissue. *J Cell Physiol*. 2013;228(10):2024-2036.

44. Wong KE, Szeto FL, Zhang W, et al. Involvement of the vitamin D receptor in energy metabolism: regulation of uncoupling proteins. *Am J Physiol Endocrinol Metab*. 2009;296(4):E820-828.

45. Wong KE, Kong J, Zhang WS, et al. Targeted expression of human vitamin D receptor in adipocytes decreases energy expenditure and induces obesity in mice. *J Biol Chem*. 2011;286(39):33804-33810.

46. Xu Y, Lou Y, Kong J. VDR regulates energy metabolism by modulating remodeling in adipose tissue. *Eur J Pharmacol*. 2019;865:172761.

47. Haussler MR, Whitfield GK, Haussler CA, et al. The nuclear vitamin D receptor: biological and molecular regulatory properties revealed. *J Bone Miner Res*. 1998;13(3):325-349.

48. Whitfield GK, Remus LS, Jurutka PW, et al. Functionally relevant polymorphisms in the human nuclear vitamin D receptor gene. *Mol Cell Endocrinol*. 2001;177(1–2):145-159.

49. Morrison NA, Qi JC, Tokita A, et al. Prediction of bone density from vitamin D receptor alleles. *Nature*. 1994;367(6460):284-287.

50. Carling T, Ridefelt P, Hellman P, et al. Vitamin D receptor polymorphisms correlate to parathyroid cell function in primary hyperparathyroidism. *J Clin Endocrinol Metab*. 1997;82(6):1772-1775.

51. Verbeek W, Gombart AF, Shiohara M, et al. Vitamin D receptor: no evidence for allele-specific mRNA stability in cells which are heterozygous for the Taq I restriction enzyme polymorphism. *Biochem Biophys Res Commun*. 1997;238(1):77-80.

52. Yamamoto H, Miyamoto K, Li B, et al. The caudal-related homeodomain protein Cdx-2 regulates vitamin D receptor gene expression in the small intestine. *J Bone Miner Res*. 1999;14(2):240-247.

53. Tokita A, Matsumoto H, Morrison NA, et al. Vitamin D receptor alleles, bone mineral density and turnover in premenopausal Japanese women. *J Bone Miner Res*. 1996;11(7):1003-1009.

54. Gomez Alonso C, Naves Diaz ML, Diaz-Corte C, et al. Vitamin D receptor gene (VDR) polymorphisms: effect on bone mass, bone loss and parathyroid hormone regulation. *Nephrol Dial Transplant*. 1998;13(suppl 3):73-77.

55. Bell NH, Morrison NA, Nguyen TV, et al. ApaI polymorphisms of the vitamin D receptor predict bone density of the lumbar spine and not racial difference in bone density in young men. *J Lab Clin Med*. 2001;137(2):133-140.

56. Uitterlinden AG, Fang Y, Van Meurs JB, et al. Genetics and biology of vitamin D receptor polymorphisms. *Gene*. 2004;338(2):143-156.

57. Li YC, Kong J, Wei M, et al. 1,25-Dihydroxyvitamin D(3) is a negative endocrine regulator of the renin-angiotensin system. *J Clin Invest*. 2002;110(2):229-238.

58. Xiang W, Kong J, Chen S, et al. Cardiac hypertrophy in vitamin D receptor knockout mice: role of the systemic and cardiac renin-angiotensin systems. *Am J Physiol Endocrinol Metab*. 2005;288(1):E125-132.

59. Bouillon R, Carmeliet G, Verlinden L, et al. Vitamin D and human health: lessons from vitamin D receptor null mice. *Endocr Rev*. 2008;29(6):726-776.