

# Association of Vitamin D Receptor Gene Polymorphisms with Metabolic Syndrome in Chinese Children

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**Purpose:** To investigate the association between vitamin D receptor (VDR) gene polymorphisms and vitamin D deficiency, overweightness/obesity, and metabolic syndrome (MetS) in a cohort of Han children residing in Hangzhou, China.

**Patients and Methods:** This study assessed 106 overweight/obese and 86 healthy (control) children. Five single-nucleotide polymorphisms (SNPs) in the VDR gene, namely, TaqI (rs731236 T > C), ApaI (rs7975232 C > A), BsmI (rs1544410 G > A), FokI (rs2228570 G > A), and Cdx2 (rs11568820 G > A), were genotyped by sequencing the total polymerase chain reaction products. The distributions of different genotypes and alleles were compared among different groups.

**Results:** The serum 25-hydroxyvitamin D (25(OH)D) concentration was significantly lower in overweight/obese children, while the AA genotype of ApaI SNP exhibited higher frequencies in the overweight/obese group than in the control. Furthermore, children with the ApaI AA genotype showed higher levels of Glu-60min, Glu-90min, Glu-120min and triglyceride. The AA genotype of FokI SNP was significantly associated with MetS. However, no association was observed between the five VDR SNPs and the risk of vitamin D deficiency.

**Conclusion:** VDR ApaI polymorphisms appear to be correlated with overweightness/obesity and glucose intolerance. FokI polymorphisms may be linked to a higher susceptibility toward MetS in Chinese children.

**Keywords:** genotype, glucose intolerance, 25-hydroxyvitamin D, allele frequencies, adiposity

## Introduction

Recent years have seen a rapid increase in the incidence of obesity in children and adolescents, leading to widespread concern.<sup>1,2</sup> Similar to that observed in adults, obesity in children can cause metabolic abnormalities including hypertension, dyslipidemia, and insulin resistance, clustered as metabolic syndrome (MetS).<sup>3</sup> MetS is associated with an increased risk of cardiovascular disease,<sup>3</sup> which is currently, the leading cause of death in China.<sup>4</sup>

Vitamin D, an essential nutrient, is primarily involved in calcium homeostasis and bone mineralization. Previous studies have demonstrated the multiple roles of vitamin D, such as in adipogenesis, glucose-insulin homeostasis, and cell growth,<sup>5</sup> which partly explains the close association between vitamin D insufficiency and various chronic diseases, including obesity.<sup>6</sup> Previous studies have found an inverse association between vitamin D levels and adiposity or MetS in children of varied

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ethnicity,<sup>7–9</sup> possibly due to changes in inflammatory factors,<sup>10</sup> parathyroid hormone production,<sup>11</sup> or activation of  $\text{Ca}^{2+}$ -dependent calpain.<sup>12</sup> However, these speculations remain unconfirmed. A better understanding of the underlying mechanism of vitamin D action may help with the early identification and prevention of MetS and associated complications in the pediatric population.

The primary circulating form of vitamin D is 25-hydroxyvitamin D (25(OH)D). When converted to its biologically active form, 1,25-dihydroxyvitamin D (1,25(OH)<sub>2</sub>D), it binds to the vitamin D receptor (VDR) in tissues and triggers a series of effects. VDR is typically expressed in several non-calcium-regulating cell types, including islet  $\beta$ -cells and adipocytes.<sup>13,14</sup> 1,25(OH)<sub>2</sub>D<sub>3</sub>/VDR signaling suppress brown adipocyte differentiation and mitochondrial respiration.<sup>15</sup> VDR knockout mice exhibited atrophy of the mammary adipose compartment compared to their wild-type littermates, and were found to be resistant to high-fat, diet-induced weight gain,<sup>16</sup> suggesting that genetic variability in VDR may affect adiposity and glucose metabolism. In humans, the VDR gene is located on chromosome 12q13.11. Among its numerous single-nucleotide polymorphisms (SNPs), five variants, namely, TaqI (rs731236 T > C), ApaI (rs7975232 C > A), BsmI (rs1544410 G > A), FokI (rs2228570 G > A) and Cdx2 (rs11568820 G > A), have been previously reported.<sup>6</sup> Several studies have demonstrated the association of these VDR gene polymorphisms with vitamin D deficiency, obesity, and glucose intolerance in children and adolescents. However, these results remain inconsistent, while only limited studies have focused on the relationship between VDR and MetS. The present study aimed to determine whether VDR gene polymorphisms are associated with vitamin D deficiency, overweightness/obesity, and MetS, in a cohort of Han children aged 6–14 years in China.

## Patients and Methods

### Study Participants

In total, 106 overweight/obese children (mean age:  $10.83 \pm 2.31$  years old) and 86 healthy-weight counterparts (mean age:  $10.15 \pm 2.53$  years old), aged 6–14 years, were enrolled in the present study. All the participants were recruited from the Department of Pediatrics, The First Affiliated Hospital, College of Medicine, Zhejiang University, between October 2018 and February 2019. Healthy-weight, overweightness, and obesity were defined

as the  $\geq 5$ th to <85th,  $\geq 85$ th to <95th, and  $\geq 95$ th percentile of body mass index (BMI) for age and gender, respectively, with categories demarcated using the cut-off points recommended by the Group of China Obesity Force.<sup>17</sup> The present study was approved by the Ethics Committee of The First Affiliated Hospital, College of Medicine, Zhejiang University and performed in accordance with the principles of the Declaration of Helsinki. A written informed consent was obtained from all participants and their legal guardians.

The exclusion criteria were as follows: presence of acute infectious or inflammatory processes, oral corticosteroid use, and a clinical history of chronic diseases, including tumors, as well as chromosome, liver or kidney, immune, rheumatic, and endocrine diseases (except for obesity).

### Anthropometric Assessment

The anthropometric data of all subjects were obtained in light clothing and without shoes by a trained staff member. Weight and height were measured using a digital scale to the nearest 0.1 kg, and a stadiometer to the nearest 0.1 cm, respectively. Waist circumference (WC) was measured using a tape placed at the midpoint level, between the lower intercostal border and the anterior superior iliac supine, while the subject remained in standing position and gently exhaled. Blood pressure was measured following a 5-min resting period, using an automatic digital device with appropriate cuff sizes. BMI and waist-to-height ratio (WHtR) were calculated as weight (kg)/height (m)<sup>2</sup>, and waist (cm)/height (cm), respectively. The Z-score for BMI-for-age (BMI z-score) was calculated according to the World Health Organization guidelines.<sup>18</sup> Abdominal obesity was defined as  $\geq 90$ th percentile of WC for age and gender, in accordance with the Chinese cut-off points.<sup>19</sup>

### Laboratory Analysis

Blood samples were collected from participants following an overnight fasting period. Total cholesterol (TC), triglyceride (TG), and high-density lipoprotein cholesterol (HDL-C) levels were measured using standard enzymatic methods. Low-density lipoprotein cholesterol (LDL-C) was calculated using the Friedewald formula. Fasting blood glucose (FBG) was measured using the glucose oxidase method. All measurements were conducted using an automatic biochemistry analyzer (Mindray BS-380). The 25(OH)D serum levels were measured by

electrochemiluminescence immunoassay using a clinical chemistry analyzer (Elecys 2010, Roche Diagnostics).

In total, 93 subjects with a BMI  $\geq$  90th percentile were randomly selected to undergo the oral glucose tolerance test (OGTT). Following overnight fasting, these subjects were given a glucose solution of 1.75 g per kg of body weight (capped at 75 g) orally, in the morning of the test day. Venous blood was collected at 0, 30, 60, 90, and 120 min following ingestion, for the measurement of glucose levels. The area under the curve (AUC) that correlated with the glucose levels and tested time-points during the procedure was calculated using the trapezoidal rule.

MetS was defined by abdominal obesity, and the presence of two or more other clinical features (elevated TG, low HDL-C, high blood pressure, or increased plasma glucose) in children older than 10 years, according to the guidelines of the International Diabetes Federation.<sup>20</sup> Vitamin D deficiency was diagnosed at 25(OH)D  $<$  20 ng/mL.<sup>21</sup>

## Genotyping

DNA was extracted from blood leukocytes using a genomic DNA kit (Generay Biotech Ltd., Shanghai, China), according to the manufacturer's instructions, and stored at  $-20^{\circ}\text{C}$  until further analysis. VDR TaqI, ApaI, BsmI, FokI, and Cdx2 genotyping was conducted by sequencing the total polymerase chain reaction (PCR) products. Five different fragments, which consisted of recognized VDR variants, were amplified using PCR. The primer sets used are shown in [Supplement Table 1](#). PCR amplification was conducted in a 50- $\mu\text{L}$  reaction volume, which consisted of 5  $\mu\text{L}$  of 10 $\times$  PCR buffer, 4  $\mu\text{L}$  of dNTPs (2.5 mM), 4  $\mu\text{L}$  of each primer (10  $\mu\text{M}$ ), 4  $\mu\text{L}$  of the genomic DNA template (20–100 ng), 0.4  $\mu\text{L}$  of Taq polymerase (5 U/ $\mu\text{L}$ ), and water. The cycling conditions were as follows: denaturation at  $95^{\circ}\text{C}$  for 5 min, followed by 30 cycles of denaturation at  $95^{\circ}\text{C}$  for 30 s, annealing at  $62.5^{\circ}\text{C}$  for 30 s, and extension at  $72^{\circ}\text{C}$  for 30 s. A final extension step followed at  $72^{\circ}\text{C}$  for 5 min. The resulting labeled PCR products were subjected to capillary electrophoresis on an ABI 3730 Genetic Analyzer sequencing system (Applied Biosystems).

## Statistical Analysis

Statistical analysis was performed using the IBM SPSS Statistics software (version 23.0). Data were tested for normality using the Kolmogorov–Smirnov test. Continuous variables were expressed as mean  $\pm$  standard deviation (SD). Student's *t*-tests were used to compare continuous variables between the two groups. In instances where the variables were not normally distributed, Mann–

Whitney *U*-tests were applied. The distribution of VDR polymorphisms was tested for the Hardy–Weinberg equilibrium (HWE) using the Chi-square ( $\chi^2$ ) test. This test was also used to detect any significant differences in the frequencies of the alleles and genotypes for each SNP between the overweight/obese group and healthy controls.

The associations of ApaI variants with different biological characteristics were evaluated using the one-way analysis of variance (ANOVA) test. The associations of VDR SNP polymorphisms with vitamin D deficiency, MetS, and its components were evaluated using multivariate logistic regression analysis, including age as a covariate. Odds ratio (OR) was used to evaluate the association strength. Linkage disequilibrium (LD) and haplotypes were calculated using the SHEsis software platform (<http://analysis.bio-x.cn/myAnalysis.php>).<sup>22</sup>  $P < 0.05$  was considered statistically significant.

## Results

### Characteristics of Study Population and Genotyping

The clinical and biochemical characteristics of the tested subjects are presented in [Table 1](#). The BMI z-score, WHtR, systolic blood pressure (SBP), diastolic blood pressure (DBP), as well as TG, TC, HDL-C and LDL-C levels were higher in the overweight/obese group compared to that in the controls ( $P < 0.05$  for each tested parameter), while serum 25 (OH)D concentration was lower in the overweight/obese group ( $P = 0.001$ ). Furthermore, 68 (63.0%) subjects from the overweight/obese group and 41 (47.7%) subjects from the control group presented vitamin D deficiency ( $P = 0.022$ ).

The sequencing results are shown in [Supplement Figure 1](#). The results of genotyping and allele distribution of all five SNPs are summarized in [Table 2](#).

### Association of VDR Polymorphisms with Overweightness/Obesity

The distribution of SNP genotypes and allele frequencies in overweight/obese children and healthy controls is presented in [Table 3](#). The whole population was maintained in the HWE for all five SNPs ( $P > 0.05$ ). The ApaI AA genotype was significantly associated with an increased risk of overweightness/obesity (OR = 6.16, 95% CI: 1.33–28.62,  $P = 0.010$ ). Although the frequency of the A allele was higher than that of the C allele in the overweight/obese group, the difference was not statistically significant (OR = 1.45,  $P > 0.05$ ). No significant difference was observed between the

**Table 1** The Clinical and Biochemical Profiles of Subjects

	Overweightness/Obesity	Controls	P
N (male/female)	106 (70/36)	86 (60/26)	0.583 <sup>a</sup>
Age (years)	10.83 ± 2.31	10.15 ± 2.53	0.050
BMI z-score	2.41 (1.78, 3.15)	-0.47 (-0.79, -0.19)	<0.001 <sup>b</sup>
Wht	0.58 (0.54, 0.61)	0.42 (0.40, 0.44)	<0.001 <sup>b</sup>
SBP (mmHg)	117.56 ± 9.77	106.30 ± 8.03	<0.001
DBP (mmHg)	69.22 ± 8.75	65.70 ± 7.47	0.004
FBG (mmol/L)	5.30 ± 0.74	5.17 ± 0.30	0.100
TG (mmol/L)	1.26 ± 0.67	0.68 ± 0.26	<0.001
TC (mmol/L)	4.11 ± 0.62	3.79 ± 0.56	<0.001
LDL-C (mmol/L)	2.33 ± 0.54	1.76 ± 0.41	<0.001
HDL-C (mmol/L)	1.27 ± 0.27	1.45 ± 0.24	<0.001
25(OH)D (ng/mL)	18.44 ± 6.50	21.83 ± 7.32	0.001
N (Vitamin D deficiency)	68	41	0.022 <sup>a</sup>

**Notes:** The values are presented as mean ± SD or median (interquartile range). <sup>a</sup>P values were calculated using the Chi-square ( $\chi^2$ ) test. <sup>b</sup>P values were calculated using the Mann-Whitney U-test.

**Abbreviations:** BMI, body mass index; WhtR, waist-to-height ratio; SBP, systolic blood pressure; DBP, diastolic blood pressure; FBG, fasting blood glucose; TG, triglycerides; TC, total cholesterol; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; 25(OH)D, 25-hydroxyvitamin D; SD, standard deviation.

control and overweight/obese groups in the distribution of alleles or genotypes of the other four SNPs.

## Association of ApaI SNP Genotypes and Biochemical Parameters

The biological characteristics of different ApaI SNP genotypes are shown in [Supplement Figure 2](#), which shows a significant association between the AA genotype and higher levels of Glu-60min ( $P = 0.021$ ), Glu-90min ( $P = 0.035$ ), Glu-120min

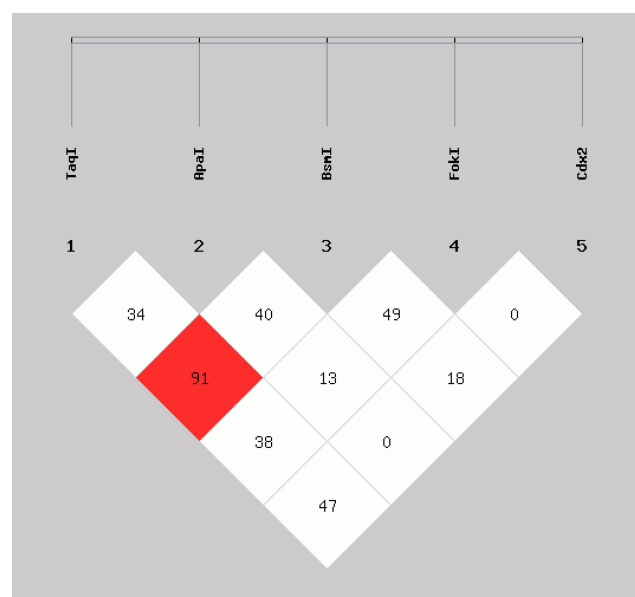
( $P = 0.025$ ), AUC ( $P = 0.048$ ) and TG ( $P = 0.026$ ). A higher FBG ( $P = 0.080$ ) and a lower HDL ( $P = 0.177$ ) level were also observed, although not statistically significant. No associations were observed with the remaining four SNPs (data not shown).

## Association of VDR Polymorphisms with MetS

Individual comparisons between the recessive/dominant model of VDR SNPs and MetS are presented in [Table 4](#). After adjusting for age, the ApaI SNP AA genotype was found to be significantly associated with increased risk of abdominal obesity (OR = 4.01,  $P = 0.039$ ) and elevated plasma glucose levels (OR = 3.88,  $P = 0.020$ ). Logistic regression analysis also confirmed the positive association of the FokI SNP AA genotype with susceptibility to MetS (OR = 3.07,  $P = 0.045$ ), in the age-adjusted recessive model. However, no association was found between FokI and the individual MetS components ( $P > 0.05$ ). Furthermore, there was no significant association between Cdx2, TaqI, and BsmI genotypes and the risk of vitamin D deficiency, MetS, or MetS components ( $P > 0.05$ ). In the present study, the recessive models of TaqI and BsmI were excluded due to the rare occurrence of their minor alleles.

## Haplotype and LD Analyses of VDR Polymorphisms

[Figure 1](#) illustrates the LD in  $D'$  values among the VDR gene polymorphisms. Pair-wise tests revealed a strong LD between the TaqI and BsmI variants in the tested subjects



**Figure 1** Plot showing the linkage disequilibrium (LD) among vitamin D receptor (VDR) gene polymorphisms in  $D'$  values.

**Note:** The red square indicates high linkage disequilibrium.

**Table 2** Genotypes and Allele Frequencies of the Studied SNPs

SNPs	Alleles (A/a)	SN Location	Chr 12 Position	Patients (n)	Genotype Frequencies			Allelic Frequencies	
			(GRCh38.p12)		AA	Aa	aa	A	a
rs731236 (Taql)	T/C	exon	47,844,974	191	166 (86.5)	23 (12.0)	2 (1.0)	0.93	0.07
rs7975232 (ApaI)	C/A	intron	47,845,054	190	94 (49.0)	80 (41.7)	16 (8.3)	0.71	0.29
rs1544410 (BsmI)	G/A	intron	47,846,052	190	160 (83.3)	27 (14.1)	3 (1.6)	0.91	0.09
rs2228570 (FokI)	G/A	exon	47,879,112	180	51 (26.6)	91 (47.4)	38 (19.8)	0.54	0.46
rs11568820 (Cdx2)	G/A	promoter	47,908,762	191	61 (31.8)	88 (45.8)	42 (21.9)	0.55	0.45

**Note:** "A" represents the major allele, while "a" represents the minor allele.

**Abbreviations:** SNP, single-nucleotide polymorphism; VDR, vitamin D receptor.

**Table 3** Distribution of SNP Genotypes and Allele Frequencies Between Overweight/Obese Children and Healthy Controls

SNPs	Genotype/Alele	Overweightness/Obesity	Controls	P <sup>a</sup>	OR	95% CI	P <sup>b</sup>
Taql	TT	92 (86.8%)	74 (87.1%)	0.547	I	0.37–2.10	0.769
	TC	12 (11.3%)	11 (12.9%)		0.88		
	CC	2 (1.9%)	0		/		
	T	196 (92.5%)	159 (93.5%)		I	0.53–2.62	0.683
	C	16 (7.5%)	11 (6.5%)		1.18		
ApaI	CC	50 (47.6%)	44 (51.8%)	0.984	I	0.51–0.68	0.798
	CA	41 (39.0%)	39 (45.9%)		0.93		
	AA	14 (13.3%)	2 (2.4%)		6.16		
	C	141 (67.1%)	127 (74.7%)		I	0.92–2.27	0.108
	A	69 (32.9%)	43 (25.3%)		1.45		
BsmI	GG	92 (86.8%)	68 (81.0%)	0.603	I	0.22–1.16	0.105
	GA	11 (10.4%)	16 (19.0%)		0.51		
	AA	3 (2.8%)	0		/		
	G	195 (92.0%)	152 (90.5%)		I	0.41–1.69	0.605
	A	17 (8.0%)	16 (9.5%)		0.83		
FokI	GG	28 (26.7%)	23 (30.7%)	0.976	I	0.55–2.18	0.796
	GA	52 (49.5%)	39 (52.0%)		1.10		
	AA	25 (23.8%)	13 (17.3%)		1.58		
	G	108 (51.4%)	85 (56.7%)		I	0.81–1.88	0.326
	A	102 (48.6%)	65 (43.3%)		1.24		
Cdx2	GG	33 (31.1%)	28 (32.9%)	0.631	I	0.55–2.06	0.848
	GA	49 (46.2%)	39 (45.9%)		1.07		
	AA	24 (22.6%)	18 (21.2%)		1.13		
	G	115 (54.2%)	95 (55.9%)		I	0.71–1.60	0.749
	A	97 (45.8%)	75 (44.1%)		1.07		

**Notes:** <sup>a</sup>P-value for HWE test in both groups. <sup>b</sup>P-value for OR.

**Abbreviations:** HWE, Hardy-Weinberg equilibrium; OR, odds ratio; SNP, single-nucleotide polymorphism; CI, confidence interval.

( $D' = 0.90$ ,  $r^2 = 0.67$ ), but not between other VDR SNPs. Haplotype analysis results between the overweight/obese and healthy groups are presented in Table 5, with no association detected.

## Discussion

The present study investigated the association of five VDR SNPs with overweightness/obesity and MetS in Han

Chinese children. Our findings revealed that ApaI was positively linked to overweightness/obesity, as well as glucose and TG levels. In addition, the results indicated an association between FokI and MetS. To the best of our knowledge, this is the first study to report the association of common VDR polymorphisms with all MetS components, as well as with vitamin D levels, in Chinese children.



**Table 4** SNP Genotype Distributions and Risk Assessments for Metabolic Syndrome and Its Components with Vitamin D Deficiency, Using Genetic Models

SNPs/Models	Genotypes	Metabolic Syndrome		Abdominal Obesity		Increased Plasma Glucose		High Blood Pressure		Low HDL-C		Elevated TG		Vitamin D Deficiency	
		OR	P	OR	P	OR	P	OR	P	OR	P	OR	P	OR	P
ApaI															
Dominant	CC	1		1		1		1		1		1		1	
	CA+AA	0.73	0.540	1.01	0.975	0.57	0.161	2.83	0.137	0.85	0.745	0.74	0.481	1.21	0.519
Recessive	CC+CA	1		1		1		1		1		1		1	
	AA	1.85	0.406	4.01	0.039	3.88	0.020	1.18	0.878	1.46	0.641	1.59	0.496	2.84	0.120
FokI															
Dominant	GG	1		1		1		1		1		1		1	
	AA+GA	0.91	0.865	1.45	0.278	1.06	0.901	0.29	0.056	1.15	0.802	1.03	0.949	0.97	0.931
Recessive	GG+GA	1		1		1		1		1		1		1	
	AA	3.07	0.045	1.32	0.476	1.59	0.305	1.48	0.585	1.51	0.477	1.64	0.316	0.51	0.069
Cdx2															
Dominant	GG	1		1		1		1		1		1		1	
	AA+GA	0.53	0.215	1.05	0.886	0.67	0.310	0.85	0.803	1.48	0.485	0.56	0.193	0.74	0.351
Recessive	GG+GA	1		1		1		1		1		1		1	
	AA	1.07	0.912	1.18	0.640	1.38	0.466	0.31	0.269	1.63	0.372	1.40	0.495	0.60	0.148
TaqI															
Dominant	TT	1		1		1		1		1		1		1	
	CC+TC	0.78	0.758	0.80	0.615	0.74	0.644	2.07	0.390	0	0.998	2.80	0.057	0.93	0.869
BsmI															
Dominant	GG	1		1		1		1		1		1		1	
	AA+GA	1.08	0.910	0.51	0.119	0.78	0.674	1.42	0.671	0.31	0.270	2.00	0.189	0.93	0.857

**Note:** P-values were calculated using the multivariate logistic regression analysis model, which included age.

**Abbreviations:** SNP, single-nucleotide polymorphism; HDL-C, high-density lipoprotein cholesterol; TG, triglycerides.

ApaI, located on the 3' untranslated region of the *VDR* gene, may influence mRNA stability and expression status of the *VDR* protein.<sup>23</sup> Herein, we demonstrated that the risk of overweightness/obesity was higher in 6- to 14-year-

old children with the *VDR* ApaI SNP AA genotype. Compared with the CA and CC genotypes, children with the ApaI AA genotype also showed higher levels of Glu-60min, Glu-90min, Glu-120min, and AUC. The ApaI

**Table 5** Haplotype Analysis Results Between Overweight/Obese and Healthy Children

Haplotypes (TaqI-ApaI-BsmI -FokI-Cdx2)	Frequency n (%)		OR	95% CI	P
	Overweight/Obesity Children	Controls			
T-A-G-G-A	14.22 (6.8)	5.48 (3.9)	1.808	0.246	0.657 ~ 4.973
T-A-G-G-G	16.63 (8.0)	13.91 (9.8)	0.786	0.529	0.371 ~ 1.665
T-A-G-A-A	17.08 (8.2)	5.36 (3.8)	2.261	0.101	0.834 ~ 6.133
T-A-G-A-G	7.77 (3.7)	6.98 (4.9)	0.740	0.572	0.259 ~ 2.109
T-C-G-G-A	29.50 (14.2)	22.57 (15.9)	0.859	0.620	0.471 ~ 1.566
T-C-G-G-G	32.78 (15.8)	33.44 (23.5)	0.590	0.056	0.342 ~ 1.017
T-C-G-A-A	28.04 (13.5)	20.37 (14.3)	0.915	0.778	0.492 ~ 1.701
T-C-G-A-G	43.98 (21.1)	19.89 (14.0)	1.637	0.097	0.912 ~ 2.938

**Abbreviations:** OR, odds ratio; CI, confidence interval.

polymorphism may therefore be closely correlated with obesity and glucose metabolism in children. Our findings are consistent with those of previous studies. A study among healthy Han Chinese adults found that the ApaI T allele (G > T) was associated with an increase in body fat percentage and triceps skin fold thickness.<sup>24</sup> A meta-analysis reported an association between insulin resistance-related diseases and the VDR ApaI variant in Asians.<sup>25</sup> A study conducted in Lebanon reported that, in young men, the TT genotype for ApaI (T > G) presented a higher BMI and WC,<sup>26</sup> while a study from Egypt showed that obese women carrying mutant ApaI alleles exhibited a significantly higher level of insulin resistance.<sup>27</sup> In Saudi Arabia, a study of 131 young female students showed that carrying the ApaI A allele protected against increased BMI.<sup>28</sup> However, several studies have reported contradictory results, indicating that the ApaI polymorphism in obese children and adolescents has no correlation to BMI, FBG, fasting insulin, or AUC.<sup>29,30</sup> This disparity may be due to existing heterogeneity among populations, age range, sample size and experimental methodology, or due to other genetic or environmental factors. Further research is therefore warranted to determine the relationship between VDR and glucose metabolism in children.

We showed that the risk of MetS significantly increased with the FokI AA genotype, suggesting that VDR gene polymorphisms may play a role in MetS development in children. Consistent with our results, a study that assessed 190 Egyptian adults reported that the occurrence of the TT genotype for VDR FokI (C > T) was significantly more frequent in diabetics with MetS, compared to those without MetS as well as healthy controls.<sup>31</sup> However, a study conducted on Thai adults detected no association between VDR FokI variants and the risk of MetS.<sup>32</sup> Similarly, a study conducted on 697 Russian middle-aged women determined that the VDR gene polymorphisms, BsmI, ApaI, TaqI, and FokI were not associated with an increased risk of MetS.<sup>23</sup> Notably, the subjects in these studies were all adults, and that the definition for MetS varied among different countries. Results from these investigations may therefore be limiting in terms of comparability. Studies that determine the relationship between VDR FokI and MetS remain limited, especially in children. We consequently scrutinized those that focused on the association between FokI and cardio-metabolic markers, such as T2DM and dyslipidemia. Two studies have confirmed the significant association between FokI polymorphisms and T2DM,<sup>33,34</sup> while another

showed that the FokI variant may increase the susceptibility to dyslipidemia in the Chinese Han population.<sup>35</sup> FokI is located on the second exon of the *VDR* gene, and the FokI polymorphism creates an alternative transcription start site, leading to a VDR protein with 3 amino acids shorter than the one without the polymorphism.<sup>36</sup> This change consequently influences its activity, thereby lowering its transcriptional activation effectiveness and altering the functional properties of the receptor,<sup>37</sup> which may partly explain these findings.

As evidence from clinical studies indicates that vitamin D deficiency is linked to the higher susceptibility of cardiovascular risk factors,<sup>7,38</sup> the relationship between VDR and MetS may be partly mediated by vitamin D; vitamin D supplement or a life style intervention (eg increasing sun-exposure duration) may be useful in mitigating metabolic abnormalities. Our study also found that serum 25 (OH)D level was significantly lower in the overweight/obese group. However, correlation does not infer causality; hence, additional research is warranted to elucidate the biological mechanisms and to determine whether vitamin D supplementation is beneficial. Furthermore, no association was found between VDR SNP polymorphisms and the risk of vitamin D deficiency, suggesting that other mechanisms may connect VDR and MetS. Consistent with this speculation, Oh et al reported that the deletion of macrophage VDR in mice promoted insulin resistance and accelerated atherosclerosis, suggesting that VDR dysfunction might result in insulin resistance.<sup>39</sup> Insulin resistance is essential for MetS and the pathogenesis of its individual metabolic components,<sup>3</sup> and this may partly explain the association between VDR and MetS.

## Limitation of This Study

There are several limitations in our study. First, the sample size was relatively small for a genetic polymorphisms study, especially for TaqI and BsmI SNPs, which have a relatively low minor allele frequency, possibly producing false-negative results. Second, information regarding the diet habit, vitamin D supplement intake, and sun-exposure duration of the study subjects was not collected, which may reduce the comparability of vitamin D status among the different groups. Finally, the onset of puberty may influence certain glucose metabolic parameters, eg serum insulin levels; however, our study did not include Tanner stage records or serum insulin measurements. Therefore, a comprehensive survey is warranted to confirm the observed association using a larger sample size.

## Conclusion

The present study demonstrated that the VDR ApaI SNP may significantly influence obesity and glucose metabolism in pediatric population, while FokI SNP variants may be associated with a susceptibility to MetS. This may be independent of the serum vitamin D status, although the incidence of vitamin D deficiency was more common among overweight/obese children. Further investigations are warranted to determine the association between VDR SNPs and vitamin D status, MetS and metabolic outcomes, and the underlying mechanisms, through multi-center randomization studies with larger cohorts of children.

## Abbreviations

VDR, vitamin D receptor; MetS, metabolic syndrome; 25 (OH)D, 25-hydroxyvitamin D; SNP, single-nucleotide polymorphism; 1,25(OH)<sub>2</sub>D, 1,25-dihydroxyvitamin D; BMI, body mass index; WC, waist circumference; WHtR, waist-to-height ratio; FBG, fasting blood glucose; TC, total cholesterol; TG, triglycerides; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; OGTT, oral glucose tolerance test; AUC, area under the curve; SD, standard deviation; OR, odds ratio; LD, linkage disequilibrium; SBP, systolic blood pressure; DBP, diastolic blood pressure; PCR, polymerase chain reaction; HWE, Hardy–Weinberg equilibrium.

## Data Sharing Statement

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

## Ethics Approval and Informed Consent

The present study was approved by the Ethics Committee of The First Affiliated Hospital, College of Medicine, Zhejiang University and performed in accordance with the principles of the Declaration of Helsinki. A written informed consent was obtained from all participants and their legal guardians.

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## Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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## Disclosure

The authors report no conflicts of interest in this work.

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