Original Article

Genetic variations of vitamin D receptor gene in metabolic syndrome and related diseases in the Thai population

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Background and Objectives: The genetic variations of vitamin D receptor (VDR) have revealed its association with the risk of metabolic syndrome (MetS). In Thailand, evidence of this association has not been obtained. Thus, this study aimed to investigate the association of VDR gene polymorphism with MetS and related diseases as well as the possible linkage disequilibrium (LD) and haplotypes of VDR in Thai adults. **Methods and Study Design:** Four single nucleotide polymorphisms (SNPs) of VDR gene, rs2228570, rs1544410, rs7975232 and rs731236, were genotyped using PCR-RFLP method in 259 MetS and 261 control groups. **Results:** Genotypes AA of rs1544410, TG of rs7975232 and TG+TT of rs7975232 were significantly associated with an increased risk of MetS [OR 10.8 (2.07–56.1), p=0.005], [OR 1.83 (1.16–2.87), p=0.009] and [OR 1.78 (1.17–2.72), p=0.007], respectively, using GG as a reference. Moreover, genotype AA of rs1544410 showed a strong association compared with GG+AG [OR 11.4 (2.20–59.2), p=0.004]. Diseases related to MetS also had significant associations with two SNPs of the VDR gene (rs1544410 and rs7975232). In addition, LD among rs1544410, rs7975232 and rs731236 was detected. Haplotype CATT significantly increased the risk of MetS [OR 4.32 (1.32–14.1), p=0.016], although haplotype TGGT reduced the risk [OR 0.68 (0.48–0.98), p=0.042]. **Conclusions:** The SNPs rs1544410 and rs7975232 were mainly implicated in the increased risk of MetS in the Thai population. LD and haplotypes of VDR gene related to MetS were also discovered. These SNPs of VDR gene are remarkable genetic factors involved in the development of MetS.

Key Words: vitamin D receptor, single nucleotide polymorphism, metabolic syndrome, Thai population, haplotype

INTRODUCTION

As one of the major public health problems around the world, metabolic syndrome (MetS) is a cluster of metabolic abnormalities, including insulin resistance/impaired glucose intolerance (increased fasting glucose levels), central obesity, hypertension and atherogenic dyslipidemia (increased triglyceride and decreased high-density lipoprotein cholesterol levels), all of which accelerate the progression of cardiovascular diseases (CVDs) and type 2 diabetes mellitus (T2DM).[1-3] The global prevalence of MetS is continuously increasing. Currently, an estimated 20%–25% of the global adult population has MetS,[4] with a prevalence rate of 10%–30% among East and Southeast Asian populations.[5] In Thailand, the fourth Thai National Health Examination Survey (NHES IV) 2008-2009 identified a MetS prevalence of 23.2%.[6] The explicit causal factors of MetS are the current topic of research, although insulin resistance and central obesity were initially considered as significantly contributing factors.[7,8] In addition, some studies suggested that vitamin D levels are correlated with the presence of MetS.[9,10] However, the action of vitamin D is mediated by binding the active metabolite $1,25(OH)_2D_3$ to a specific vitamin D receptor (VDR).[11]

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VDRs are members of the nuclear receptor family that functions as a transcriptional activator of many genes.[12]

Recently, VDR gene polymorphisms have been implicated in enhancing susceptibility to MetS. DNA polymorphisms that were often reported for VDR gene included rs2228570 (C/T, *Fok*I) in exon 2, rs1544410 (A/G, *Bsm*I) and rs7975232 (T/G, *Apa*I) located in intron 8 and rs731236 (C/T, *Taq*I) located in exon 9.[13] Several studies found that variations in the VDR gene were associated with the risk of this clustered disorder.[14-19] However, results are inconsistent; this may be due to the variation in ethnicity between study populations. Currently, the molecular mechanism of VDR polymorphisms in the MetS pathological landscape remains unclear. Moreover, the four aforementioned single nucleotide polymorphisms (SNPs) of the VDR gene and their associations with MetS as well as linkage disequilibrium (LD) and haplotypes in the Thai population have not been reported.

Therefore, the aim of this study was to investigate the association of these four VDR gene polymorphisms (rs2228570, rs1544410, rs7975232 and rs731236) with MetS and its components in Thai adults as well as diseases related to MetS. Moreover, possible LD and haplotypes of the VDR gene were determined.

METHODS

Subjects

Thai volunteers aged between 18 and 87 years living in suburban and urban areas of Bangkok, Thailand were enrolled in this study. Participants were randomly selected from the outpatient department of a medical centre that focused on the endocrine and metabolic systems by experienced medical doctors associated with our project. All participants provided informed consent and completed questionnaires regarding demographic information as well as previous medical history. Patients with severe illness, e.g. renal failure, liver failure, heart failure and stroke, or people who heavily smoked or with a history of alcoholism were excluded from the study. A total of 520 participants were classified into either healthy control (261 subjects) or MetS (259 subjects) groups. MetS subjects were identified based on the International Diabetes Federation (IDF) criteria.[20] A person considered to have MetS must have central obesity (defined as waist circumference ≥90 cm for Asian men and ≥80 cm for Asian women) plus any two of the following four factors:

- Increased triglyceride levels: ≥150 mg/dL (1.7 mmol/L) or on a specific treatment for this lipid abnormality
- Decreased HDL cholesterol levels: <40 mg/dL (1.03 mmol/L) in males and <50 mg/dL (1.29 mmol/L) in females or on a specific treatment for this lipid abnormality
- Increased blood pressure: systolic BP ≥ 130 or diastolic BP ≥85 mmHg or on a treatment for previously diagnosed hypertension
- Increased fasting plasma glucose levels: ≥100 mg/dL (5.6 mmol/L) or previously diagnosed with type 2 diabetes

The study protocol was approved by the Ethical committee of the Faculty of Tropical Medicine, Mahidol University (MUMT 2016-003-01).

Anthropometric measurements

Body weight, total body fat and visceral fat were measured using Karada scan (Omron, Japan). Height was measured using a standard height measurement scale. Waist circumference was measured using a flexible standard tape. BMI was calculated as weight in kilograms divided by height in squared meters (kg/m^2). In addition, pulse and blood pressure were measured using a sphygmomanometer (UDEX-Twin, Japan) and the average value of three times measurement was calculated.

Biochemical measurements

Biochemical measurements, including glucose, glycated haemoglobin (HbA1C), total cholesterol, HDL-C, LDL-C and triglyceride levels, were determined using an automatic analyzer (ARCHITECT ci8200, Abbott). The radioimmunoassay (RIA) method was used to measure serum insulin levels. Homeostatic model assessment of insulin resistance (HOMA-IR) was calculated using the following equation: HOMA-IR = (Glucose × Insulin)/405, wherein the unit of glucose was mg/dL.

Genotyping

Genomic DNA was extracted from peripheral leukocytes using a DNA extraction kit (FlexiGene® DNA kit, Qiagen). After extraction, the quantity and purity of DNA samples were measured using a Nanodrop ND-1000 spectrophotometer (version 3.3).

Genetic variations in the VDR gene (rs2228570, rs1544410, rs7975232 and rs731236) were determined using the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method. The Primer 3 plus program was used to design three pairs of PCR primers, as shown in Table 1. PCR was performed in 50 µL reactions containing forward and reverse primers, dNTPs, buffer and $MgCl_2$, Tag DNA polymerase and DNA template using a C1000TM Thermal cycler (Bio-Rad, USA).

Table 1. Primer sequences and annealing temperatures of VDR gene variants

SNPs	Primer pair forward and reverse: 5' to 3'	Annealing temperature (°C)	Product size (bp)
rs2228570	5' CACTGACTCTGGCTCTGACC 3' 5' GAGATGTGAAAAATGCAAGGGCTC 3'	57.5	284
rs1544410	5' CCTCACTGCCCTTAGCTCTG 3' 5' AACCAGCGGAAGAGGTCAAG 3'	63.0	280
rs7975232 rs731236	5' ACAGATGTGAAGGCTGGTGG 3' 5' GGTCGGCTAGCTTCTGGATC 3'	61.4	463

VDR: vitamin D receptor; SNPs: single nucleotide polymorphisms; bp: base pair.

Initially, samples were heated for 5 minute (min) at 95°C, followed by 34 cycles for 30 second (s) at 95°C, 30 s at 57.5–63°C (Table 1) and 50 s at 72°C, with an extension step of 5 min at 72°C. PCR products of rs2228570, rs1544410 and rs7975232 were digested with the restriction endonucleases *Fok*I, *Hha*I and *Apa*I, respectively, for 3 hour (h) at 37°C. For rs731236, the product was digested for 3 h at 65°C with *Taq*I endonuclease. The digested fragments were analysed by gel electrophoresis using a low range DNA ladder as a marker (Figure 1). Finally, approximately 10% of the study population was randomly selected to confirm the DNA sequencing (Macrogen Inc., Seoul, South Korea).

Statistical analysis

Statistical analysis was performed using the SPSS v.18.0 software. The distribution of data was tested by the Kolmogorov–Smirnov test. Descriptive statistics such as median, inter quartile range (IQR), frequency and percentage were presented to describe the general characteristics of study participants. The Mann–Whitney U test was used to compare the control and MetS groups, whereas the Kruskal–Willis test was applied to compare the genotype of each SNPs with anthropometric variables and biochemical parameters. Odds ratios (ORs) and 95% confidence intervals (CI) were computed by binary logistic regression to test the association between genetic variation with MetS and its related diseases. The chi-square (χ^2) test was used to determine the Hardy–Weinberg equilibrium for the genotypes of these four SNPs. LD and haplotype analysis was performed using the SNPStats web tool.[21] A *p*-value of less than 0.05 was considered statistically significant.

RESULTS

General characteristics of study population

General characteristics of our study populations in the control and MetS groups are presented in Table 2. The median age was 37 (range 28–44) years in the control group, whereas it was 58 (range 51–67) years in the MetS group. Waist circumference, BMI, percentage of total body fat, level of visceral fat, blood pressure, pulse, fasting glucose, HbA1c, total cholesterol, triglyceride, insulin and HOMA-IR levels were significantly higher ($p<0.001$) and HDL-C and LDL-C levels were lower ($p<0.001$) in the MetS group compared with those in the control group. Four VDR polymorphisms, rs2228570 (in exon 2), rs1544410 and rs7975232 (in intron 8) and rs731236 (in exon 9), were investigated in all 261 control and 259 MetS participants via the PCR-RFLP technique. The bands of different variants of the VDR gene are shown in Figure 1. Each variant was confirmed by DNA se-

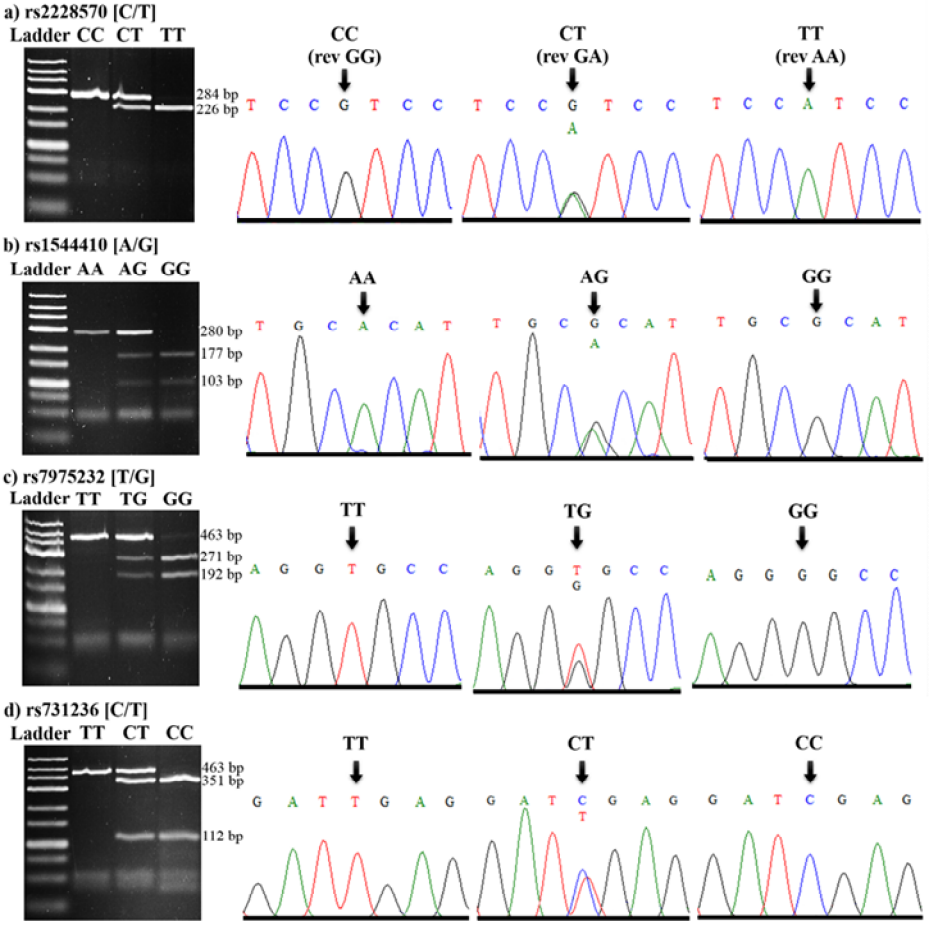

Figure 1. Digestion fragment patterns and DNA sequences of VDR gene polymorphisms. a(Bands of different genotypes and matched DNA sequences of rs2228570. b(Bands of different genotypes and matched DNA sequences of rs1544410. c(Bands of different genotypes and matched DNA sequences of rs7975232. d(Bands of different genotypes and matched DNA sequences of rs731236. Low range ladder was used as a ladder. rev: reverse strain.

Table 2. General characteristic of study population

Variables	Control (n=261)	MetS (n=259)	*p*-value
Gender (M/F)	47/214	85/174	-
Age (years)	37 (28–44)	58 (51–67)	<0.001
Waist circumference (cm)	78 (72–85)	95 (90–101)	<0.001
BMI (kg/m^2)	22.1 (20.2-24.0)	28.3 (25.4-30.9)	<0.001
Total body fat (%)	28.2 (24.4–31.8)	34.8 (29.6–38.5)	<0.001
Visceral fat (level)	5 (3–7)	14 (10–18)	<0.001
Systolic BP (mmHg)	110 (101–120)	131 (121–142)	<0.001
Diastolic BP (mmHg)	67 (60–76)	77 (68–85)	<0.001
Pulse (beats/min)	75 (69–84)	81 (73–90)	<0.001
Glucose (mg/dL)	87 (82–94)	121 (109–137)	<0.001
HbA1c (%)	5.1 (4.9–5.3)	6.3 (5.8–6.9)	<0.001
Total cholesterol (mg/dL)	200 (182–221)	169 (151–196)	<0.001
Triglyceride (mg/dL)	80 (59–109)	149 (110–188)	<0.001
LDL-C (mg/dL)	128 (107–145)	103 (86–128)	<0.001
HDL-C (mg/dL)	64 (54–76)	46 (41–54)	<0.001
Insulin (μU/mL)	11.8 (8.8–14.9)	16.4 (12.0–22.9)	<0.001
HOMA_IR	2.60 (1.91–3.32)	4.83 (3.56–7.25)	<0.001

M: men; F: women; BMI: body mass index; BP: blood pressure; HbA1c: haemoglobin A1c; LDL-C: low density lipoprotein-cholesterol; HDL-C: high density lipoprotein-cholesterol; HOMA-IR: homeostasis model assessment of insulin resistance.
Data are presented as median)interquartile range(.
$p<0.05$ was considered to be statistically significant.

quencing. Genotypic distributions of all polymorphisms in controls were in agreement with the Hardy–Weinberg equilibrium (p=0.859, 0.216, 0.091 and 0.864, respectively) (Table 3).

Association of VDR polymorphisms with MetS

As displayed in Table 4, the results showed statistical significance between genetic variations of VD*R* and MetS status, adjusted for age and sex. We introduced three models, including codominant (wild type versus heterozygous variant and wild type versus homozygous variant), dominant (wild type versus variants) and recessive (wild type with heterozygous variant versus homozygous variant). In the codominant model of the control group, the genotypes of VDR polymorphisms mostly showed high frequencies in the wild type, whereas those of rs2228570 showed the highest frequency in the heterozygous genotype. In MetS participants, the SNPs rs1544410 and rs731236 demonstrated high frequencies in wild type, whereas the others (rs2228570 and rs7975232) demonstrated high frequencies in the heterozygous genotype. In addition, genotype AA of rs1544410 and TG of rs7975232 were significantly associated with an increased risk of MetS [OR 10.8 (2.07–56.1), p=0.005] and [OR 1.83 (1.16–2.87), p=0.009], respectively, in the codominant model. Genotype AA of rs1544410 also strongly increased risk of MetS in the recessive model [OR 11.4 (2.20–59.2), p=0.004]. Moreover, the dominant model TG+TT of rs7975232 was significantly associated with MetS [OR 1.78 (1.17–2.72), p=0.007]. Anthropometric and biochemical parameters of all participants were compared according to genotypes of each VDR polymorphism (Table 5). Level of visceral fat was significantly different among the genotypes of rs2228570, whereas waist circumference, BMI, level of visceral fat, fasting glucose, HbA1c, total cholesterol, triglyceride, LDL-C and HDL-C levels were significantly different between genotypes of rs1544410. There were no statistically significant differences in any parameters regarding the rs7975232 and rs731236 genotypes.

Table 3. Genotype distribution of VDR polymorphisms among control group in the Hardy–Weinberg equilibrium

SNPs	Genotype	Observed	Expected	χ^2	*p*-value
rs2228570	TT	69 (26.4%)	68.3 (26.1%)	0.031	0.859
	CT	129 (49.5%)	130.4 (50%)		
	CC	63 (24.1%)	62.3 (23.9%)		
rs1544410	GG	216 (82.8%)	214.3 (82.1%)	1.53	0.216
	AG	41 (15.7%)	44.4 (17%)		
	AA	4 (1.5%)	2.3 (0.9%)		
rs7975232	GG	126 (48.3%)	120 (46%)	2.86	0.091
	TG	102 (39.1%)	114 (43.7%)		
	TT	33 (12.6%)	27 (10.3%)		
rs731236	TT	227 (87%)	227.2 (87%)	0.029	0.864
	CT	33 (12.6%)	32.6 (12.5%)		
	CC	1 (0.4%)	1.2 (0.5%)		

Table 4. Risk and association of each VDR polymorphisms with MetS

SNPs	Genotype†	Control	Mets	OR (95% CI)	*p*-value
rs2228570					
a:	TT	69 (26.4%)	55 (21.2%)	Reference	
	CT	129 (49.5%)	144 (55.6%)	1.23 (0.74–2.05)	0.430
	CC	63 (24.1%)	60 (23.2%)	1.09 (0.60–1.99)	0.780
b:	TT	69 (26.4%)	55 (21.2%)	Reference	
	CT+CC	192 (73.6%)	204 (78.8%)	1.19 (0.73–1.93)	0.490
c:	TT+CT	198 (75.9%)	199 (76.8%)	Reference	
	CC	63 (24.1%)	60 (23.2%)	0.95 (0.58–1.54)	0.820
rs1544410					
a:	GG	216 (82.8%)	205 (79.2%)	Reference	
	AG	41 (15.7%)	31 (11.9%)	0.66 (0.36–1.21)	0.181
	AA	4 (1.5%)	23 (8.9%)	10.8 (2.07–56.1)	0.005
b:	GG	216 (82.8%)	205 (79.2%)	Reference	
	AG+AA	45 (17.2%)	51 (20.8%)	1.14 (0.67–1.94)	0.630
c:	GG+AG	257 (98.5%)	236 (91.1%)	Reference	
	AA	4 (1.5%)	23 (8.9%)	11.4 (2.20–59.2)	0.004
rs7975232					
a:	GG	126 (48.3%)	97 (37.5%)	Reference	
	TG	102 (39.1%)	122 (47.1%)	1.83 (1.16–2.87)	0.009
	TT	33 (12.6%)	40 (15.4%)	1.65 (0.86–3.14)	0.128
b:	GG	126 (48.3%)	97 (37.5%)	Reference	
	TG+TT	135 (51.7%)	162 (62.5%)	1.78 (1.17–2.72)	0.007
c:	GG+TG	228 (87.4%)	219 (84.6%)	Reference	
	TT	33 (12.6%)	40 (15.4%)	1.23 (0.67–2.24)	0.510
rs731236					
a:	TT	227 (87%)	216 (83.4%)	Reference	
	CT	33 (12.6%)	41 (15.8%)	1.38 (0.75–2.54)	0.308
	CC	1 (0.4%)	2 (0.8%)	0.90 (0.08–10.2)	0.931
b:	TT	227 (87%)	216 (83.4%)	Reference	
	CT+CC	34 (13%)	43 (16.6%)	1.35 (0.74–2.45)	0.330
c:	TT+CT	260 (99.6%)	257 (99.2%)	Reference	
	CC	1 (0.4%)	2 (0.8%)	0.86 (0.08–9.78)	0.900

†Data was analysed by binary logistic regression and adjusted for age and sex.
(a) Codominant model analysis (wild type vs heterozygous variants and wild type vs homozygous variants).
(b) Dominant model analysis (wild type vs variants).
(c) Recessive model analysis (wild type + heterozygous variants vs homozygous variants)
$p<0.05$ was considered statistically significant.

VDR polymorphisms and risk of related diseases

To discover the association between VDR polymorphism and the diseases related to MetS, genotypes of VDR polymorphisms were compared to diseases such as diabetes, pre-diabetes, hypertension, lipid abnormalities (high cholesterol, high triglyceride, high LDL-C, or low HDL-C) and obesity, as shown in Table 6. These diseases were diagnosed by a clinician, and some participants in the MetS group were treated with medication. Genotype AA of rs1544410 illustrated the increased risk of diabetes [OR 6.83 (2.35–19.8), $p<0.001$], prediabetes [OR 3.22 (1.07–9.67), $p=0.037$], hypertension [OR 3.41 (1.15–10.1), $p=0.028$], lipid abnormality [OR 3.15 (1.31–7.57), $p=0.010$], and obesity [OR 2.71 (1.10–6.65), $p=0.030$]. In addition, the rs7975232 homozygous variant (TT genotype) showed higher risk of diabetes [OR 2.30 (1.23–4.31), $p=0.009$], whereas a combination of rs7975232 TG and TT genotypes demonstrated significant association with diabetes [OR 1.74 (1.13–2.67), $p=0.011$] and prediabetes [OR 1.63 (1.02–2.62), $p=0.043$].

LD and haplotypes of VDR

LD parameters were generated to determine the association of these four SNPs using a LD test using the SNPStats web tool.[21] Pairwise D' values between four studied SNPs were represented by the LD pattern shown in Table 7. The SNP rs7975232 displayed strong LD with both rs1544410 and rs731236 (D'>0.90, $p<0.001$). A median association was also present between rs1544410 and rs731236 (D'=0.79, $p<0.001$), whereas weak linkage with any of the other SNPs was found with respect to rs2228570. Table 8 shows the haplotype analysis of VDR polymorphisms. There were sixteen patterns of four SNP haplotypes that were possible. However, eight patterns of haplotypes were commonly detected. The CGGT pattern (ordered as rs2228570 (C/T), rs1544410 (A/G), rs7975232 (T/G) and rs731236 (C/T), respectively) was the most frequently found haplotype in this study, which was used as a reference haplotype. Haplotype TGGT significantly reduced the risk of MetS with OR 0.68 (0.48–0.98) and $p=0.042$. In addition, an increased risk of MetS was shown in haplotype CATT [OR 4.32 (1.32–14.1), $p=0.016$]. Other haplotypes did not show any effect on MetS risk.

DISCUSSION

Several studies demonstrated the association between VDR polymorphisms and MetS in various ethnicities. A study of the Northern Chinese showed that a variant of rs1544410 acted as a risk factor for MetS using GG with

Table 5. Comparison of anthropometric and biochemical parameters according to genotypes of VDR polymorphisms

	rs2228570				rs1544410			
	TT	CT	CC	*p*-value	GG	AG	AA	*p*-value
Age (years)	44 (33-58)	50 (36-61)	49 (34-59)	0.265	47 (34-59)	48 (35-61)	55 (50-61)***	0.042
Waist circumference (cm)	85 (78-95)	89 (80-95)	87 (78-95)	0.201	87 (79-95)	83 (77-93)	94 (87-101)***	0.004
BMI (kg/m^2)	24.2 (21.3-28.0)	25.3 (22.2-29.0)	24.8 (21.5-29.2)	0.171	24.8 (21.5-28.9)	23.3 (21.8-27.1)	28.7 (26.0-31.2)***	0.001
Total body fat (%)	30.6 (26.0-35.7)	31.0 (27.8-36.2)	29.5 (26.3-35.9)	0.268	30.7 (26.7-36.0)	30.0 (26.7-33.8)	33.7 (28.7-37.1)	0.214
Visceral fat (level)	8 (4-11)	9 (5-15) *	8 (4-14)	0.024	8 (5-14)	7 (4-14)	13 (9-18)***	0.003
Systolic BP (mmHg)	118 (103-132)	122 (110-133)	120 (105-132)	0.174	121 (106-132)	117 (105-132)	126 (118-133)	0.217
Diastolic BP (mmHg)	73 (61-83)	73 (64-82)	70 (64-79)	0.318	72 (63-81)	73 (63-84)	74 (64-84)	0.697
Pulse (beats/min)	79 (72-87)	79 (69-88)	78 (70-85)	0.863	79 (71-87)	75 (69-84)	82 (70-91)	0.265
Glucose (mg/dL)	96 (86-121)	99 (87-122)	95 (87-121)	0.682	97 (86-122)	94 (87-117)	116 (100-137)***	0.002
HbA1c (%)	5.8 (5.1-6.7)	5.7 (5.2-6.5)	5.7 (5.2-6.4)	0.944	5.7 (5.2-6.5)	5.5 (5.1-6.1)	6.2 (5.5-6.8)**	0.039
Total cholesterol (mg/dL)	191 (166-210)	185 (162-213)	188 (164-215)	0.706	188 (165-212)	191 (169-216)	163 (136-195)***	0.036
Triglyceride (mg/dL)	107 (67-156)	111 (76-165)	102 (78-150)	0.242	110 (73-161)	95 (75-146)	131 (106-191)***	0.008
LDL-C (mg/dL)	121 (100-140)	118 (91-141)	116 (94-143)	0.663	119 (96-141)	120 (95-144)	94 (76-127)***	0.046
HDL-C (mg/dL)	55 (47-69)	53 (44-65)	55 (44-69)	0.134	54 (44-67)	57 (50-67)	50 (44-58)**	0.035
Insulin (μU/mL)	13.0 (9.7-17.4)	13.3 (10.3-18.2)	14.8 (9.9-21.2)	0.348	13.6 (10.0-18.7)	12.7 (9.9-15.4)	13.6 (10.2-17.6)	0.386
HOMA-IR	3.28 (2.30-4.64)	3.33 (2.45-5.23)	3.60 (2.23-5.80)	0.530	3.34 (2.38-5.24)	2.96 (2.32-4.36)	4.00 (3.10-5.08)	0.067
	rs7975232				rs731236			
	GG	TG	TT	*p*-value	TT	CT	CC	*p*-value
Age (years)	46 (34-59)	48 (35-61)	51 (37-59)	0.297	48 (34-60)	50 (38-60)	51 (49-57)	0.486
Waist circumference (cm)	87 (78-95)	86 (79-95)	87 (78-96)	0.857	87 (78-95)	88 (78-95)	90 (70-116)	0.953
BMI (kg/m^2)	24.2 (20.9-28.9)	24.9 (22.0-28.7)	25.7 (22.1-29.1)	0.349	24.8 (21.5-28.9)	25.0 (22.3-28.2)	28.6 (23.1-32.7)	0.549
Total body fat (%)	30.2 (26.5-35.9)	31.3 (26.9-36.0)	30.9 (26.9-36.4)	0.751	30.6 (26.7-36.0)	31.0 (27.1-35.6)	34.0 (23.6-39.4)	0.909
Visceral fat (level)	8 (5-15)	8 (4-13)	10 (5-15)	0.341	8 (5-14)	10 (5-14)	10 (6-12)	0.876
Systolic BP (mmHg)	121 (105-132)	120 (110-132)	121 (109-130)	0.499	120 (106-132)	126 (111-132)	126 (97-131)	0.629
Diastolic BP (mmHg)	71 (63-81)	73 (64-82)	73 (65-80)	0.554	72 (64-81)	71 (60-84)	75 (72-83)	0.675
Pulse (beats/min)	78 (71-86)	79 (70-87)	77 (68-89)	0.931	78 (71-86)	80 (69-89)	93 (56-105)	0.675
Glucose (mg/dL)	96 (86-119)	99 (87-121)	101 (91-134)	0.143	97 (86-122)	105 (91-118)	116 (100-135)	0.218
HbA1c (%)	5.6 (5.2-6.4)	5.8 (5.2-6.5)	5.9 (5.2-6.7)	0.523	5.8 (5.2-6.6)	5.6 (5.2-6.2)	7.3 (5.5-7.4)	0.239
Total cholesterol (mg/dL)	189 (163-217)	187 (165-206)	177.5 (154-225)	0.511	187 (165-213)	188 (159-215)	176 (167-258)	0.944
Triglyceride (mg/dL)	112 (72-155)	102 (75-157)	127 (83-182)	0.078	111 (75-164)	98 (75-150)	126 (100-139)	0.668
LDL-C (mg/dL)	119 (96-144)	117 (95-135)	116 (88-143)	0.536	118 (95-141)	118 (90-142)	116 (110-181)	0.775
HDL-C (mg/dL)	55 (44-68)	54 (45-66)	54 (45-64)	0.833	54 (44-66)	56 (48-65)	48 (45-62)	0.565
Insulin (μU/mL)	13.4 (10.3-18.7)	13.9 (10.3-18.6)	12.9 (9.2-17.7)	0.393	13.6 (10.0-19.1)	13.1 (9.9-15.9)	17.4 (16.4-21.2)	0.228
HOMA-IR	3.34 (2.38-5.33)	3.53 (2.44-5.17)	3.21 (2.16-4.81)	0.783	3.34 (2.40-5.37)	3.34 (2.36-4.32)	5.23 (4.70-5.80)	0.272

BMI: body mass index; BP: blood pressure; HbA1c: haemoglobin A1c; TG: triglyceride; LDL-C: low density lipoprotein-cholesterol; HDL-C: high density lipoprotein-cholesterol; HOMA-IR: homeostasis model assessment of insulin resistance.

Data are presented as median (interquartile range) and analysed using the Kruskal–Wallis test.

p-value was statistically significant when <0.05. *Significant for genotype TT, **Significant for genotype GG, ***Significant for both genotype GG and AG.

Table 6. Association between VDR polymorphisms and related diseases of MetS

Diseases		rs2228570					
		CT		CC		CT + CC	
		OR (95% CI)	*p*-value	OR (95% CI)	*p*-value	OR (95% CI)	*p*-value
Diabetes	TT Reference	1.07 (0.63-1.82)	0.809	1.19 (0.64-2.21)	0.588	1.11 (0.67-1.85)	0.681
Pre-Diabetes		1.29 (0.71-2.35)	0.403	0.99 (0.49-2.02)	0.984	1.25 (0.72-2.20)	0.431
Hypertension		1.31 (0.72-2.38)	0.373	1.07 (0.53-2.15)	0.858	1.29 (0.74-2.25)	0.376
Lipid abnormality		1.53 (0.83-2.81)	0.174	1.08 (0.53-2.24)	0.827	1.36 (0.76-2.44)	0.296
Obesity		1.43 (0.78-2.64)	0.249	1.46 (0.72-2.97)	0.292	1.44 (0.80-2.60)	0.223
Diseases		rs1544410					
		AG		AA		AG + AA	
		OR (95% CI)	*p*-value	OR (95% CI)	*p*-value	OR (95% CI)	*p*-value
Diabetes	GG Reference	0.86 (0.46-1.62)	0.646	**6.83 (2.35-19.8)**	**<0.001**	1.56 (0.92-2.64)	0.101
Pre-Diabetes		0.45 (0.22-0.94)	0.033	**3.22 (1.07-9.67)**	**0.037**	0.88 (0.49-1.59)	0.671
Hypertension		0.49 (0.24-1.00)	0.050	**3.41 (1.15-10.1)**	**0.028**	0.93 (0.52-1.67)	0.811
Lipid abnormality		0.70 (0.33-1.47)	0.348	**3.15 (1.31-7.57)**	**0.010**	1.18 (0.67-2.09)	0.571
Obesity		0.63 (0.29-1.35)	0.232	**2.71 (1.10-6.65)**	**0.030**	1.04 (0.58-1.87)	0.887
Diseases		rs7975232					
		TG		TT		TG + TT	
		OR (95% CI)	*p*-value	OR (95% CI)	*p*-value	OR (95% CI)	*p*-value
Diabetes	GG Reference	1.58 (0.99-2.50)	0.054	**2.30 (1.23-4.31)**	**0.009**	**1.74 (1.13-2.67)**	**0.011**
Pre-Diabetes		1.56 (0.93-2.61)	0.095	1.59 (0.78-3.24)	0.200	**1.63 (1.02-2.62)**	**0.043**
Hypertension		1.50 (0.90-2.50)	0.124	1.65 (0.82-3.33)	0.164	1.60 (0.99-2.55)	0.051
Lipid abnormality		1.31 (0.79-2.18)	0.302	1.57 (0.80-3.10)	0.190	1.33 (0.83-2.13)	0.232
Obesity		1.11 (0.68-1.83)	0.677	1.10 (0.54-2.22)	0.796	1.11 (0.69-1.77)	0.666
Diseases		rs731236					
		TC		CC		TC + CC	
		OR (95% CI)	*p*-Value	OR (95% CI)	*p*-Value	OR (95% CI)	*p*-Value
Diabetes	TT Reference	0.88 (0.48-1.61)	0.684	3.29 (0.28-38.4)	0.342	0.94 (0.53-1.68)	0.833
Pre-Diabetes		1.35 (0.69-2.63)	0.381	0.52 (0.04-6.51)	0.608	1.27 (0.66-2.45)	0.473
Hypertension		1.42 (0.73-2.75)	0.305	0.55 (0.04-6.85)	0.639	1.33 (0.70-2.56)	0.387
Lipid abnormality		0.83 (0.42-1.61)	0.574	0.00 (0.00)	0.999	0.77 (0.40-1.50)	0.450
Obesity		0.95 (0.50-1.83)	0.886	1.78 (0.15-20.7)	0.645	0.98 (0.52-1.86)	0.961

Data was analysed by binary logistic regression adjusted age and sex.
$p<0.05$ was considered statistically significant.

Table 7. Pairwise linkage disequilibrium of four SNPs in VDR gene

SNP (D')	rs2228570	rs1544410	rs7975232	rs731236
rs2228570	-	0.072	0.040	0.107
rs1544410	-	-	0.986*	0.793*
rs7975232	-	-	-	0.974*
rs731236	-	-	-	-

Linkage disequilibrium was analysed using SNPStats program.[21]
*Significant when $p<0.05$.

AG genotypes as a reference.[19] Similarly, our result found that homozygous variant (AA) of rs1544410 has a 10.8- and 11.4-fold increased risk of MetS compared to wild type (GG) and wild type with heterozygous (GG+AG) genotypes, respectively. Moreover, our results also demonstrated that rs1544410 was significantly associated with lipid abnormalities (i.e. total cholesterol, LDL-C, HDL-C and triglyceride levels), hyperglycemia with increased HbA1c levels and the increasing trend of central obesity parameters (waist circumference, BMI and visceral fat), all of which are systematically linked to the development of this clustered syndrome. This was consistent with previous studies, which reported that rs1544410 was related to the components of MetS such as lipid profiles,[14,22] glucose levels[23] and waist circumference.[15]

Likewise, the relationship between polymorphisms of rs7975232 and MetS was also presented in this study. Heterozygous variant (TG) and combined variants (TG+TT) increased the risk of developing MetS by 1.83- and 1.78-fold, respectively. Although the increased risk of MetS by rs7975232 has not been previously reported, there was a study which found that genotype TG of rs7975232 was a potential risk factor of high blood pressure.[14] Nevertheless, some participants with MetS in our study were treated with medication, which may partially explain the non-significant difference between any genotype of rs7975232 and all parameters (anthropometric and biochemical). Furthermore, the significant difference observed in rs1544410 with anthropometric and biochemical parameters might be because of the striking increase in the risk of MetS (approximately 10-fold).

The genetic variation of VDR rs731236 in this population showed neither significant relation to MetS nor anthropometric and biochemical variables, comparable to a study of the Saudi Arabian population.[14] In contrast,

Table 8. Genotype distribution of VDR polymorphisms among control group in the Hardy–Weinberg equilibrium

Haplotype†	Control (Freq.)	MetS (Freq.)	OR	95% CI	*p*-value
CGGT	0.326	0.348		Reference	
TGGT	0.352	0.257	0.68	0.48–0.98	0.042
TGTT	0.106	0.152	1.36	0.88–2.10	0.170
CGTT	0.114	0.078	0.68	0.38–1.19	0.180
CATC	0.038	0.034	0.60	0.23–1.52	0.280
TATC	0.021	0.034	1.38	0.56–3.36	0.480
CATT	0.011	0.043	4.32	1.32–14.1	0.016
TATT	0.024	0.033	0.96	0.40–2.29	0.920
Rare	0.008	0.022	2.70	0.82–8.90	0.100

Freq: frequency.

Haplotype analysis was conducted using SNPStats program.[21]

†Haplotype patterns were ordered as rs2228570, rs1544410, rs7975232 and rs731236, respectively.

$p<0.05$ was considered statistically significant.

several studies reported significant differences among genotypes of rs2228570 concerning BMI, insulin (insulin and HOMA-IR) and some lipid profiles.[16,17,19] Our study only found significant association of rs2228570 with visceral fat, which may be due to ethnic variation. Thus, only the A allele of rs1544410 and T allele of rs7975232 were considered as risk alleles for MetS in this Thai population.

In addition, several studies also exhibited the relationship of genetic variation of VDR with diseases related to MetS. An association of rs7975232 and rs731236 of VDR gene was shown to be linked to gestational diabetes mellitus in Iranian population.[24] Rs7975232 polymorphism was associated with a significantly higher glucose intolerance in a nondiabetic Caucasian population that comprised part of the Rancho Bernardo cohort.[25] SNP rs1544410 was associated with increased susceptibility to T2DM in the Chinese Han population[26] and with an increased risk of T1DM in the East Asian population.[27] Relationships of VDR gene polymorphisms with hypertension, lipid profiles and obesity have also been reported.[15,22,28,29] Consistent with the existing data of increased MetS risk, our results presented herein highlighted that the homozygous variant of rs1544410 is associated with an increased risk of diseases related to MetS, including diabetes, pre-diabetes, hypertension, lipid abnormalities and obesity; however, variants of rs7975232 increased the risk of diabetes and pre-diabetes in this population. In addition, the association with these related diseases supports the efficient progression to MetS, as mediated by rs1544410 and rs7975232.

LD results from our study agreed with other studies, which reported that SNPs at the 3' end of the VDR gene have strong LD.[14,30-33] The strong LD of rs7975232 with rs1544410 and rs731236 was observed (D'>0.80), and rs1544410 had quite a strong association with rs731236 (D'=0.79) in this Thai population. Thus, weaker linkage between rs1544410 and rs731236 may be attributed to the position of polymorphism loci. The position of these three SNPs was in the following order: rs1544410, rs7975232 and rs73126, respectively.[13]

To our knowledge, this is the first study to report the association between MetS and haplotypes of the VDR gene. As presented in Table 8, the CATT and TGGT haplotypes (a combination of rs2228570, rs1544410, rs7975232 and rs731236) were associated with higher and lower risks of MetS, respectively. The CATT haplotype included the risk alleles A of rs1544410 and T of rs7975232, which were supported with the result of association to MetS. The reduced risk effect of the TGGT haplotype might be due to the alleles T, G, G and T of rs2228570, rs1544410, rs7975232 and rs731236 being ancestral. Haplotypes of these four SNPs associated with an increased risk of vitamin D deficiency were found in the Saudi Arabian population.[14] Recently, Al-Daghri et al found a positive relationship between obesity and higher BMI upon combination of rs731236, rs1544410 and rs7975232.[29] The susceptibility haplotype of VDR (a combination of rs1544410, rs757343, rs731236 and rs739837) to T2DM was also shown regarding Chinese ethnicity.[26] As aforementioned, this is the first study that has investigated the association of VDR gene variation with MetS in Thailand. The impact of VDR polymorphism in different ethnic groups warrants further investigation.

Conclusion

In conclusion, the present study indicated the association of VDR polymorphisms with the increased risk of MetS, especially rs1544410 and rs7975232. A and T alleles of rs1544410 and rs7975232, respectively, were recommended as key risk factors of Meets and its related diseases in the Thai population. LD and haplotypes of VDR gene related to MetS in the Thai population were also discovered. These SNPs of the VDR gene are critical genetic factors involved in MetS development. These variations, once understood, could be used to identify and monitor people at greater risk of developing MetS. Furthermore, such a monitoring tool would help to reduce the incidence of MetS as well as its consequences (e.g. T2DM and CVDs). In particular, patients at risk could be educated about changes to lifestyle factors that reduce the risk of MetS, such as food intake behaviours, physical activities, smoking habits and alcohol intake.

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