Letter to the Editor

Association of Vitamin D Receptor Gene Polymorphisms with Metabolic Syndrome in Rural Areas of China*

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Metabolic Syndrome (MS) is a combination of multiple complex diseases, whose etiology is complicated and has many influencing factors. Randomized controlled trials have shown that vitamin D supplementation could delay the ameliorate symptoms of multiple chronic disease. Therefore, it is reasonable to postulate that the expression levels of vitamin D and its related gene variants might be correlated with MS. MS has always been a serious health burden worldwide, which increases the risk of cardiovascular disease and type 2 diabetes mellitus, the prevalence varies from 20% to 40% across different regions and ethnicities.

Vitamin D functions through the binding with the vitamin D receptor (*VDR*)[1] which is located on chromosome 12q12-14 (gene ID: 7421). *VDR* gene contains 12 exons and spans about 252593kb. Once 1,25-dihydroxyvitamin D [1,25(OH)2D], the active form of vitamin D, binds to *VDR*, it's biological functions were activated by modulating the transcription of downstream target genes. The expressions of more than 2,000 genes (3% of the human genome) are regulated by vitamin D signaling[2]. Several SNPs of *VDR* genes have already been associated with T2DM, insulin secretion, as well as metabolic changes related to obesity. However, to date, there is paucity in references addressing the association of *VDR* gene polymorphisms with the risk of MS in Chinese rural population. In China, rural population stands for a special group with large number, which is suffering from the growth trend of T2DM and cardiovascular disease. Therefore, the study on the risk of MS in rural cohort is especially meaningful and important.

Data were obtained from the case-control study performed between January and May 2013 in the cities of Zhengzhou and Jiaozuo, Henan Province (31°-36°N), China. Exclusion criteria: 1) patients with chronic illnesses of kidney or liver; 2) users of antiepileptic drugs or vitamin supplements; 3) pregnant or breastfeeding women. MS was diagnosed according to the IDF metabolic syndrome definition. Finally, 426 patients with MS were included in study. The controls were matched to MS group by number, sex, age (±3), socioeconomic status and residence. Thus, a total of 852 participants were included in the present analysis. A signed consent form was obtained from all participants and the ethics committee of the School of Public Health from Zhengzhou University approved the study protocol. A group of trained technicians administered a standardized questionnaire consisting of demography, lifestyle, family and diseases history and other medical issues were used to obtain the basic information of subjects. Physicians of the investigation area hospital performed anthropometric measurements, including body height, body weight, and waist and hip circumferences. Body mass index [BMI = weight (kg)/height $(m)^2$] was calculated based on measured data. Blood samples were obtained by venipuncture after an overnight fast (> 10 h) and analyzed fasting glucose (GLU), total cholesterol (TC), triglycerides (TG), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), serum levels of 25-hydroxy-vitamin D [25(OH)D]. Homeostasis model assessment of insulin resistance (HOMA-IR) and homeostatic model assessment of β-cell function (HOMA-β).

Genotyping was performed using Applied Biosystems platform (7500 FAST Real-Time PCR system; Applied Biosystems, Foster City, USA). Finally, four SNPs (rs2228570, rs2189480, rs3847987, and rs2239179) were to be studied according to the results of the pre-test (P < 0.05) and related references. Genotyping of four novel SNPs was

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performed by TaqMan probe assay on the Applied Biosystems platform (7500 FAST Real-Time PCR system; Applied Biosystems, Inc., Carlsbad, USA). The RT-PCR thermal conditions consisted of an initial denaturing at 95 °C for 10 min followed by 40 cycles of 95 °C for 15 s (denaturing) and 60 °C for 1 min (annealing). The genotyping success rates for the 4 SNPs were all > 99.5% and the concordance rates were > 99% based on 5% duplicate samples.

The EpiData 3.1 software (Inc., DK) was used for data inputting. SPSS 21.0 (Inc., Chicago, IL, USA) was used for database management and statistical analyses. Normality of the distribution of each variable was analyzed by Kolmogorov-Smirnov test. Participant characteristics were presented as mean ± SD. Logarithmic transformation was performed to obtain a normal distribution for skewed variables (GLU, TG, Ins, DBP, 25-hydroxy-vitamin D) and presented as median and quadruple spacing. Paired-sample *t* test was used for continuous variables comparison. Based on observed and expected frequency, chi-square test were used for Hardy-Weinberg equilibrium (HWE) in both case and control groups. Analyses of haplotype construction were examined using the SHEsis (http://analysis.bio-x.cn/myAnalysis.php). The linkage disequilibrium (LD) structure and haplotype interaction analysis was examined using Haploview 4.2. As the rs2239179 was in strong linkage disequilibrium (LD) with (r^2 = 0.92). Odds ratio weighted gene risk scores (OR-GRS) was used to assess the additive effects of VDR variants on the risk of MS. All *P*-values were two-tailed, and significance was defend as $P \leq 0.05$.

It indicates that the investigated population has reached the genetic balance, that is, the data of this group survey is credible. The detail information was shown in the Supplementary Tables S1 and S2 (available in www.besjournal.com).

The general characteristics and biochemical indexes of all subjects were described in Table 1. In our study, compared with controls, BMI, waist circumference, blood pressure, glucose, triglycerides and insulin resistance were significantly higher in individuals with MS ($P < 0.05$). While the levels of HDL-C, TC, and β Cell function were lower when compared to controls ($P < 0.05$). Serum 25(OH)D concentrations in MS patients were significantly lower than that in the controls.

Our finding is in line with the results of several studies, which have shown that vitamin D level is associated with MS. Altieri et al.[3] reported that vitamin D directly influenced insulin secretion in the pancreatic β-cell through increasing intracellular free calcium concentration. However, the causality between vitamin D expression and MS is uncertain.

Table 1. Characteristics of the Study Population

Variables	MS (*n* = 426)	Control (*n* = 426)	*t*	*P* Value[a]
Age (year)	54.42 ± 13.54	53.80 ± 13.48	0.67	0.502
Male/Female (*n*)	191/235	191/235	-	-
BMI (kg/m^2)	28.09 ± 3.05	24.39 ± 3.34	16.85	< 0.001
WC (cm)	95.68 ± 8.42	83.64 ± 10.07	18.80	< 0.001
SBP (mmHg)	136.04 ± 17.08	125.83 ± 17.15	8.71	< 0.001
DBP (mmHg)	86.32 ± 10.01	80.81 ± 9.32	8.32	< 0.001
GLU (mmol/L)[b]	5.43 (4.50, 7.46)	4.64 (4.07, 5.21)	8.47	< 0.001
INS (mIU/L)[b]	13.94 (10.07, 19.39)	9.64 (7.06, 13.61)	8.86	< 0.001
HOMA IR[b]	1.34 (0.84, 1.72)	0.74 (0.33, 1.14)	11.53	< 0.001
HOMA β[b]	130.61 (62.51, 247.52)	162.02 (88.26, 325.13)	-3.65	< 0.001
TG (mmol/L)[b]	2.12 (1.46, 3.12)	1.08 (0.71, 1.56)	16.69	< 0.001
TC (mmol/L)	4.73 ± 1.12	4.55 ± 0.98	2.45	0.014
HDL-C (mmol/L)	1.20 ± 0.28	1.44 ± 0.27	-13.41	< 0.001
LDL-C (mmol/L)	2.42 ± 0.84	2.52 ± 0.74	-1.90	0.058
25(OH)D (ng/mL)[b]	17.20 (14.47, 27.39)	20.07 (15.05, 39.40)	-4.26	< 0.001

Note. Data are presented as mean ± SD or quartile range; statistical significance ($P < 0.05$) is shown. *n*: number of individuals; BMI: body mass index; WC: waist circumference; SBP: systolic blood pressure; DBP: diastolic blood pressure; GLU: glucose; INS: insulin; HOMA IR: insulin resistance; HOMAβ: β Cell function; TG: triglycerides; TC: total cholesterol; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol; 25(OH)D: Serum 25-hydroxy-vitamin D. [a]The paired-sample T test for continuous variables. [b]Variables were natural log-transformed before statistical analysis.

Additionally, we observed no significantly difference among rs2228570, rs2189480, rs3847987, and rs2239179 of *VDR* polymorphic genotypes in serum vitamin D level. In a study of 1,443 Chinese from Yunnan, Li et al.[4] found that there were significant association between *VDR*-rs2228570 and serum levels of 25(OH)D, which was consistent with our findings.

None of the genotype frequencies was found to deviate from Hardy-Weinberg equilibrium in both case- and control- groups. Table 2 shows that when compared with the CC genotype, the TT of rs2228570 was associated with an increased risk of MS (P = 0.004). As for rs2189480, CC and CA genotypes were protective factors as comparing to AA (P = 0.018, P = 0.043). We also found that allele C of both rs2189480 and rs2239179 were protective factors of MS (P = 0.011, P = 0.009). In rs3847987, individuals who carried AA genotypes had a higher risk of MS than those of CC genotype (P = 0.002). Supplementary Tables S3 and S4 (available in www.besjournal.com) demonstrate the distribution of all clinical variables according to the genotypes observed in MS group and control group.

The biological actions of 1,25-dihydroxyvitamin D_3[1,25$(OH)_2D_3$] are mediated by VDR. The association of *VDR* gene polymorphisms and MS is little investigated. Our study was the first to investigate the association of rs2228570, rs2189480, rs3847987, and rs2239179 genetic variants in *VDR* to MS in Chinese rural area.

Among several genetic variants of *VDR*, rs2228570, located in the initiator codon, may lead to missense. We found that allele TT of *VDR*-rs2228570 was associated with a risk of MS, which is in accordance with the results of a few studies. In a study of 243 adults from Brazilian, Schuch NJ et al.[5] found that individuals with MS and TT-rs2228570 had lower concentrations of intact parathyroid hormone (iPTH), as well as β Cell dysfunctions, suggesting that TT genotype was associated with insulin resistance. No such conclusion could be drawn from our study. However, Zhao Y et al.[6] found that genotype TT of the rs2228570 polymorphism was correlated with lower BMI in subjects with MS, while rs2228570 was not significantly associated with MS. These discrepancies may due to the frequency distribution of the genotypes and populations difference[7]. In a study of 958 adenoma cases and 909 healthy controls from the Tennessee colorectal polyp study (TCPS), Barry EL et al.[8] found that the A allele of rs2228570 for men was positively correlated to serum adiponecin concentrations, the mechanism of which may be related to the regulating effect of vitamin D signaling on adipogenesis, and thus inhibits adipocyte differentiation[9].

The *VDR* rs2189480, rs3847987, and rs2239179 are less investigated compared with other SNPs in current studies. The locus rs2189480 is one of the single nucleotide variations (SNVs) located in intron region, which has been found in association with T2DM and cardiovascular disease. We found that genotype AA of rs3847987 was associated with MS, while genotype CC and allele C of rs2189480, allele G of rs2239179 seemed to play a protective role. The rs3847987 located in 3' untranslated region (UTR), has been found to be related to colorectal cancer[10]. The rs2239179, also located in intron region, has been reported to be significantly associated with 25(OH)D concentration on waist-hip ratio (WHR)[11]. All four SNPs are in non-coding region of the *VDR* gene. Although introns are noncoding regions, interionic SNPs can potentially affect protein synthesis by influencing RNA splicing. Moreover, introns may have their own transcription units that produce regulatory RNAs or small proteins, and thus affect the expressions of the derivates[12]. Polymorphisms at the 3' UTR of *VDR* gene are associated with its expression, possibly through modulating messenger RNA stability. Hence, the interionic SNPs may still influence the function of *VDR* gene once they are in high-linkage disequilibrium with alleles of a functional sequence variation in the 3' UTR region. There are too few studies on rs2189480, rs3847987, and rs2239179 of *VDR* gene to support our conclusions. Therefore, our finding awaits replication.

According to our study, rs2239179 and rs2189480 were in complete LD (r^2 = 0.92) (Supplementary Figure S1, available in www.besjournal.com). The frequencies values of haplotypes with a frequency over 0.03 are listed in Table 3. The haplotype A-A significantly increased the risk of MS (P = 0.009) and individuals carrying the haplotype G-C have a lower risk of MS (P = 0.014). Supplementary Table S5 (available in www.besjournal.com) shows that the risk of MS for the second grades (-0.365 ≤ GRS < -0.104) was 1.87 fold (P = 0.003) compared with the first grades (GRS ≥ -0.104).

This was the first research to analyze these four SNPs with MS in one crowd. Our results may help to further understanding of the biological significance of *VDR* polymorphisms in relation to MS, and

contribute to the identification of novel therapeutic strategies for MS. There were some limitations to this study, and therefore, the results should be evaluated with caution. The main limitation lies in the design of case-control studies, which limits the findings to the quality of the pairs between groups. The potential limitations of these data merit consideration. Therefore, our results require further investigation with larger sample sizes. And the present study did not include environmental factors, so we are not able to correlate gene-environment interactions to MS.

Table 2. Allele and Genotype Frequencies of Single Nucleotide Polymorphisms of the Vitamin D Receptor (*VDR*) Gene in the Study Population

SNPs	Genotype	MS *n*, (%)	Control *n*, (%)	χ^2	*P*	*OR*[a] (95% *CI*)	*P*[a]
rs2228570	CC	108 (25.4)	133 (31.2)	9.06	0.011	1.00	
	CT	211 (49.5)	222 (52.1)			1.12 (0.81-1.55)	0.503
	TT	107 (25.1)	71 (16.7)			1.82 (1.21-2.715)	0.004
	CT + TT	318	293			1.29 (0.95-1.75)	0.106
	C	427 (50.1)	488 (57.3)	8.78	0.030	1.00	
	T	425 (49.9)	364 (42.7)			0.77 (0.57-1.04)	0.092
rs2189480	AA	204 (47.9)	169 (39.7)	6.67	0.036	1.00	
	CA	168 (39.4)	185 (43.4)			0.73 (0.54-0.99)	0.043
	CC	54 (12.7)	72 (16.9)			0.60 (0.40-0.92)	0.018
	CC + AC	222	257			0.70 (0.53-0.93)	0.012
	A	576 (67.6)	523 (76.5)	2.99	0.084	1.00	
	C	276 (27.1)	329 (23.5)			0.70 (0.55-0.92)	0.011
rs3847987	CC	264 (62.0)	279 (65.5)	12.70	0.002	1.00	
	CA	131 (30.8)	138 (32.4)			0.98 (0.73-1.33)	0.911
	AA	31 (7.3)	9 (2.1)			3.33 (1.54-7.22)	0.002
	CA + AA	162	147			1.13 (0.85-1.51)	0.391
	C	659 (77.3)	696 (81.7)	4.93	0.026	1.00	
	A	193 (22.7)	156 (18.3)			1.17 (0.88-1.55)	0.281
rs2239179	AA	274 (64.3)	237 (55.6)	6.78	0.034	1.00	
	AG	131 (30.8)	165 (38.7)			0.66 (0.50-0.89)	0.006
	GG	21 (4.9)	24 (5.6)			0.76 (0.41-1.42)	0.393
	AG + GG	152	189			0.67 (0.51-0.89)	0.006
	A	679 (79.7)	639 (75.0)	5.36	0.021	1.00	
	G	173 (20.3)	213 (25.0)			0.69 (0.52-0.91)	0.009

Note. [a]Adjusted for age, sex, smoking, alcohol use, high-fat diet, physical activity, hypertension and the family history of obesity and diabetes.

Table 3. Association Analysis for Haplotypes of the Two Single Nucleotide Polymorphisms of the Vitamin D Receptor (*VDR*) Gene between Cases and Controls

Haplotype	MS 2*n* (%)	Control 2*n* (%)	*OR* (95% *CI*)	*P*
rs2239179-rs2189480				
AA	565.79 (66.4)	514.87 (59.9)	1.31 (1.07-1.60)	0.009
AC	113.21 (13.3)	124.13 (14.6)	0.90 (0.68-1.19)	0.457
GC	162.79 (19.1)	204.87 (24.0)	0.75 (0.59-0.94)	0.014

In conclusion, we reported that MS was highly prevalent in rural population of Henan province in China. Furthermore, our results showed that the *VDR* gene (rs2228570, rs3847987, rs2189480, and rs2239179) variants were significantly associated with MS and haplotype of AA rs2239179-rs2189480 were associated with higher MS susceptibility.

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Supplementary Table S1. Hardy-weinberg Equilibrium of Control Group

Genotype		Predictive Value	Observed Value	χ^2	*P*-value
rs2228570	CC	140	133 (31.2%)	0.815	0.665
	CT	209	222 (52.1%)		
	TT	77	71 (16.7%)		
rs2189480	AA	249	169 (39.6%)	2.037	0.361
	AC	154	185 (43.4%)		
	CC	24	72 (17.9%)		
rs3847987	CC	283	279 (64.5%)	1.665	0.435
	CA	122	138 (32.4%)		
	AA	13	9 (2.1%)		
rs2239179	AA	239	237 (55.6%)	0.262	0.877
	AG	160	165 (38.7%)		
	GG	27	24 (5.7%)		

Supplementary Table S2. Hardy-weinberg Equilibrium of Case Group

Genotype		Predictive Value	Observed Value	χ^2	*P*-value
rs2228570	CC	107	108 (25.4%)	0.019	0.991
	CT	213	211 (49.5%)		
	TT	106	107 (25.1%)		
rs2189480	AA	226	204 (47.9%)	1.541	0.463
	AC	168	168 (39.4%)		
	CC	31	54 (12.7%)		
rs3847987	CC	255	264 (62.0%)	2.968	0.227
	CA	150	131 (30.8%)		
	AA	22	31 (7.3%)		
rs2239179	AA	271	274 (64.3%)	0.482	0.807
	AG	138	131 (30.8%)		
	GG	18	21 (4.9%)		

Supplementary Table S3. The Relationships of the Vitamin D Receptor Genotypes and the Metabolic Syndrome's Components in MS Group

A. The Relationships of rs2228570, rs2189480 Genotypes and the Metabolic Syndrome's Components in MS Group

Variables	rs2228570			F/χ^2	P [a]	rs2189480			F/χ^2	P [a]
	CC	CT	TT			AA	CA	CC		
WC (cm)	95.89 ± 7.64	95.71 ± 8.97	95.40 ± 8.11	0.094	0.911	95.14 ± 8.06	96.71 ± 9.04	94.49 ± 7.49	2.225	0.109
SBP (mmHg)	138.60 ± 17.43	135.96 ± 8.97	133.63 ± 15.75	2.292	0.102	136.36 ± 17.62	135.50 ± 17.57	136.52 ± 13.28	0.141	0.868
DBP (mmHg)	86.87 ± 10.64	85.51 ± 9.40	87.34 ± 10.49	1.412	0.245	85.12 ± 9..62	86.97 ± 10.33	88.80 ± 9.99	3.530	0.030
GLU (mmol/L)[b]	6.75 (4.55-8.19)	6.34 (4.47-7.09)	6.60 (4.50-7.83)	0.857	0.652	6.56 (4.56-7.83)	6.54 (4.47-7.03)	6.24 (4.38-6.39)	0.139	0.933
TG (mmol/L)[b]	2.89 (1.51-3.74)	2.50 (1.51-3.03)	2.60 (1.33-2.95)	2.707	0.251	2.601 (1.43-3.03)	2.60 (1.53-3.11)	2.73 (1.48-3.54)	0.503	0.778
HDL-C (mmol/L)	1.16 ± 0.21	1.24 ± 0.31	1.18 ± 0.25	3.041	0.219	1.22 ± 0.256	1.19 ± 0.28	1.20 ± 0.34	2.983	0.225
25 (OH)D (ng/mL)[b]	36.09 (11.71-28.56)	40.96 (11.91-39.49)	43.30 (12.83-35.21)	2.560	0.278	40.00 (11.76-33.55)	40.23 (12.46-35.35)	41.47 (12.51-39.97)	2.622	0.270

B. The Relationships of rs3847987, rs2239179 Genotypes and the Metabolic Syndrome's Components in MS Group

Variables	rs3847987		F/χ^2	P [a]	rs2239179			F/χ^2	P [a]
	CC	CA + AA			AA	AG	GG		
WC (cm)	96.01 ± 8.30	95.40 ± 8.92	0.675	0.500	96.01 ± 8.30	95.40 ± 8.92	93.01 ± 8.42	1.340	0.263
SBP (mmHg)	136.35 ± 17.07	135.16 ± 17.72	0.647	0.518	136.35 ± 17.07	135.16 ± 17.72	137.54 ± 13.24	0.298	0.742
DBP (mmHg)	86.07 ± 9.98	86.49 ± 10.24	-0.391	0.696	86.07 ± 9.98	86.49 ± 10.24	88.38 ± 9.19	0.546	0.580
GLU (mmol/L)[b]	6.46 (4.49-7.22)	6.60 (4.50-7.84)	0.298	0.585	6.56 (4.54-7.84)	6.47 (4.46-6.97)	6.08 (4.31-7.68)	0.727	0.695
TG (mmol/L)[b]	2.57 (1.50-3.09)	2.70 (1.44 -3.26)	0.202	0.653	2.60 (1.42-3.01)	2.65 (1.47-3.33)	2.75 (1.56-3.44)	1.477	0.478
HDL-C (mmol/L)	1.20 ± 0.26	1.20 ±.0.29	0.285	0.776	1.20 ± 0.26	1.20 ± 0.29	1.24 ± 0.35	1.162	0.559
25 (OH)D(ng/mL)[b]	39.61 (12.07-37.05)	41.37 (12.11-34.84)	0.489	0.484	38.36 (12.21-32.20)	44.60 (11.83-39.23)	38.29 (12.84-46.13)	0.568	0.753

Note. Data are presented as mean ± SD or quartile range; statistical significance ($P < 0.05$) is shown. WC: Waist circumference; SBP: Systolic blood pressure; DBP: Diastolic blood pressure; GLU: glucose; TG: triglycerides; HDL-C: High-density lipoprotein cholesterol; 25(OH)D: Serum 25-hydroxy-vitamin D. [a] The paired-sample *t* test and *F* test for normal distribution continuous variables; Cruskal-Wallis *H* test for abnormal distribution continuous variables. [b]Variables were natural log-transformed before statistical analysis.

Supplementary Table S4. Relationships of the Vitamin D Receptor Genotypes and the Metabolic Syndrome's Components in Control Group

A. The Relationships of rs2228570, rs2189480 Genotypes and the Metabolic Syndrome's Components in Control Group

Variables	rs2228570			F/χ^2	P [a]	rs2189480			F/χ^2	P [a]
	CC	CT	TT			AA	CA	CC		
WC (cm)	83.87 ± 10.69	83.61 ± 10.15	83.32 ± 8.68	0.073	0.930	83.15 ± 9.85	84.49 ± 10.24	82.62 ± 10.11	1.223	0.295
SBP (mmHg)	124.90 ± 18.56	126.29 ± 16.89	126.15 ± 15.24	0.288	0.750	125.69 ± 16.27	126.30 ± 17.50	124.94 ± 18.39	0.172	0.842
DBP (mmHg)	80.56 ± 10.06	80.72 ± 9.24	81.52 ± 8.13	0.264	0.768	80.88 ± 9.10	80.80 ± 9.48	80.63 ± 9.52	0.018	0.982
GLU (mmol/L)[b]	5.15 (4.14-5.10)	5.25 (4.14-5.29)	4.95 (3.87-5.07)	3.606	0.165	5.31 (4.00-5.30)	5.08 (4.16-5.23)	5.07 (4.03-5.07)	1.320	0.517
TG (mmol/L)[b]	1.23 (0 .66-1.51)	1.38 (0.75-1.66)	1.26 (1.05-1.44)	2.035	0.361	1.30 (0.77-1.52)	1.38 (0.68-1.64)	1.20 (0.62-1.58)	1.354	0.508
HDL-C (mmol/L)	1.46 ± 0.26	1.43 ± 0.29	1.45 ± 0.26	0.377	0.686	1.44 ± 0.27	1.45 ± 0.28	1.43 ± 0.25	0.208	0.812
25 (OH)D (ng/mL)[b]	41.81 (11.91-42.37)	41.5 3 (11.89-46.13)	36.07 (11.65-30.21)	1.183	0.553	36.72 (11.35-31.24)	41.21 (12.08 -40.15)	48.83 (12.84-65.08)	7.320	0.026

B. The Relationships of rs3847987, rs2239179 Genotypes and the Metabolic Syndrome's Components in Control Group

Variables	rs3847987		t/Z	P [a]	rs2239179			F/χ^2	P [a]
	CC	CA + AA			AA	AG	GG		
WC (cm)	83.18 ± 9.90	84.53 ± 10.36	-1.313	0.190	83.27 ± 9.55	125.71 ± 16.17	80.70 ± 9.32	0.554	0.575
SBP (mmHg)	125.17 ± 17.13	127.08 ± 17.16	-1.094	0.274	84.28 ± 10.99	125.62 ± 18.90	80.49 ± 9.24	0.308	0.735
DBP (mmHg)	80.68 ± 9.09	81.05 ± 9.77	-0.386	0.700	82.93 ± 8.49	128.50 ± 13.92	84.00 ± 9.63	1.522	0.219
GLU (mmol/L)[b]	5.10 (4.05-5.16)	5.31 (4.20-5.32)	-1.610	0.107	5.27 (4.03-5.32)	5.07 (4.08-5.10)	4.86 (4.53-5.09)	1.589	0.452
TG (mmol/L)[b]	1.33 (0.68 -1.56)	1.28 (0.73-1.56)	-0.707	0.480	1.26 (0.75-1.52)	1.45 (0.69-1.69)	0.94 (0.62-1.18)	3.736	0.154
HDL-C (mmol/L)	1.44 ± 0.26	1.44 ± 0.28	0.387	0.699	1.44 ± 0.27	1.44 ± 0.27	1.45 ± 0.25	0.006	0.994
25 (OH)D(ng/mL)[b]	44.86 (11.86-49.82)	32.82 (11.64-31.00)	-1.663	0.096	40.86 (11.47-38.31)	40.37 (12.81-40.99)	41.78 (12.83-65.28)	3.444	0.179

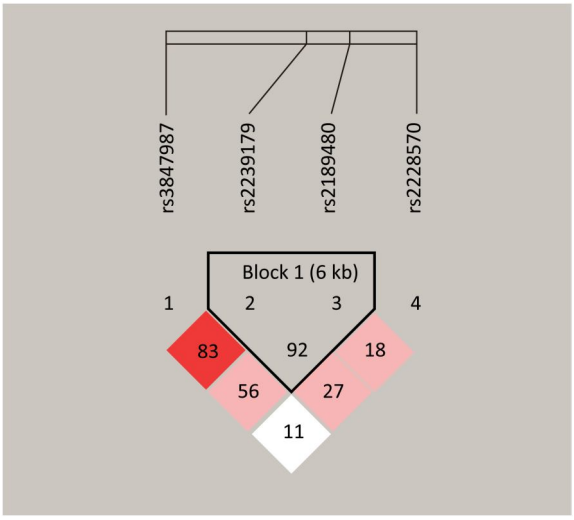
Note. Data are presented as mean ± SD or quartile range; statistical significance ($P < 0.05$) is shown. WC: Waist circumference; SBP: Systolic blood pressure; DBP: Diastolic blood pressure; GLU: glucose; TG: triglycerides; HDL-C: High-density lipoprotein cholesterol; 25(OH)D: Serum 25-hydroxy-vitamin D.

[a] The paired-sample *t* test and *F* test for normal distribution continuous variables; Cruskal-Wallis *H* test for abnormal distribution continuous variables.

[b] Variables were natural log-transformed before statistical analysis.

Supplementary Table S5. Association between GRS of Four SNPs and MS

GRS	MS 2n (%)	Control 2n (%)	*OR* (96% *CI*)	*P*
≥ -0.104	136 (31.9)	140 (32.9)	1.00	
-0.365 to -0.104	101 (23.7)	54 (12.7)	1.87 (1.23, 2.83)	0.003
-0.675 to -0.365	94 (22.1)	127 (19.8)	0.74 (0.51, 1.06)	0.100
< -0.675	95 (22.3)	105 (24.6)	0.88 (0.61, 1.28)	0.515



Supplementary Figure S1. Linkage Disequilibrium Plots with r^2 Values were generated using Haploview for the *VDR* Genes.