Letter to the Editor

Significant Polymorphisms of Vitamin D Receptor Gene (rs2189480 and rs3847987) Related to the Risk of Type 2 Diabetes in Henan Rural Area*

HAN Han[1], ZHAO Ming Xu[2], WANG Yan[1], WANG Jun[1], REN Bing Nan[3], GE Hui Na[1], WANG Teng[1], SUN Bei Bei[1], BA Yue[2], and LI Wen Jie[1,#]

Type 2 diabetes mellitus (T2DM) accounts for 90% of all diabetes cases and results in severe complications. It is a multifactorial metabolic disorder that results from a combination of resistance to insulin action and an inadequate compensatory insulin secretory response. The interaction between multiple genetic and environmental determinants has been considered to contribute to the pathogenesis of T2DM[1].

Accumulating evidence has demonstrated that vitamin D supplementation has a positive effect on glucose homeostasis and low circulating vitamin D levels are associated with an increasing risk of developing T2DM across diverse populations[2]. The 1,25-dihydroxyvitamin D [1,25(OH)D] depends on vitamin D receptor (*VDR*) to exert its biological effects. The *VDR* gene is located on chromosome 12q13.1, contains 11 exons, and has an extensive promoter region capable of generating multiple tissue-specific transcripts. Among them, FokI, BsmI, ApaI, and TaqI are the four most common single nucleotide polymorphisms (SNPs) that have been repeatedly reported to be associated with T2DM, fasting glucose levels, and insulin sensitivity[3]. However, the association between novel polymorphic loci in the *VDR* gene and T2DM remains unclear.

Currently, Henan is the most populous province in China. The prevalence of T2DM in Henan is higher than the nationwide average level[4]. There is limited published knowledge regarding the association between novel SNPs in *VDR* (rs2189480 and rs3847987) and T2DM in the rural population of China. Therefore, this study was conducted to detect the interaction of environmental, behavioral, and genetic factors with T2DM in Henan province and provide a preliminary theoretical basis on the control and prevention of T2DM.

We selected six incorporated villages from Houzhai, Zhengzhou city (34°N), and Qiaomiao, Jiaozuo city (35°N), of Henan province as the study sites. A total of 287 patients were included in the T2DM group after excluding individuals with other types of diabetes, chronic kidney disease, vitamin D supplementation, and treatment in the past 3 months. Control subjects were matched with T2DM cases by number, gender, age (±5 years), socioeconomic status, and residence. Patients with T2DM were diagnosed according to the criteria recommended by the World Health Organization (WHO, 1999) and the standard version of the 2002 American Diabetes Association guidelines[5]. A well-designed questionnaire pertaining to details regarding demography, lifestyle, family and disease history, and other medical issues was used to interview the participants person-to-person by professional investigators. Anthropometry measurements were performed by a clinician locally using comprehensive indexes, for example, stature, weight, waist circumference, and hip circumference. Body mass index (BMI), waist:hip ratio (WHR), and waist : height ratio (WHtR) were calculated based on the measured data. All subjects were asked to assemble on time after an overnight fast (> 10 h) for the collection of blood samples. Oral antidiabetic drugs, insulin, and alcohol should have been avoided for at least 12 h before blood collection. Our reserach was approved by the ethics review committee of life sciences, Zhengzhou university.

The levels of fasting glucose, total cholesterol (TC), triglycerides (TG), high-density lipoprotein

doi: 10.3967/bes2019.009

*This work was supported by grants from the National Natural Science Foundation of China [81573151, 81872626, and 81573243]; Science and Technology Foundation for Innovation Talent of Henan Province [154200510010]; and Dietary Nutrition Research and Education Foundation of Danone [DIC2013-06].

1. Department of Nutrition and Food Hygiene, College of Public Health, Zhengzhou University, Zhengzhou 450000, Henan, China; 2. Department of Environmental Hygiene, College of Public Health, Zhengzhou University, Zhengzhou 450000, Henan, China; 3. Department of Epidemiological, College of Public Health, Zhengzhou University, Zhengzhou 450000, Henan, China

cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C) were measured by an automatic biochemical analyzer (KHB, Shanghai, China). Other basic biochemical blood tests were routinely performed using standard chemical and enzymatic commercial methods in the laboratories. Serum 25-hydroxyvitamin D [25(OH)D] and $1,25(OH)_2D$ levels were measured by enzyme-linked immunosorbent assay (Sangon Biotech, Shanghai, PR China), according to the manufacturer's instructions.

Genomic DNA of leukocytes was extracted from the peripheral blood samples using the Blood DNA Kit (Bioteke Inc., Beijing, China). For this study, 24 tagging SNPs were selected from the International HapMap project and GenBank. Finally, two SNPs (rs2189480 and rs3847987) were identified for investigation. Genotyping for the two novel SNPs was performed using the 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 step at 95 °C for 10 min, followed by 40 cycles at 95 °C for 15 s (denaturing) and at 60 °C for 1 min (annealing). A random 5% of samples were repeated independently for quality control, and the success rates of both the SNPs were > 99.5%.

Data analysis was carried out using SPSS version 21.0 (Inc., Chicago, IL, USA). Normally distributed data such as age were expressed as mean ± standard deviation (SD), whereas median (interquartile range) was applied to non-normally distributed data [such as TG and 25(OH)D levels]. Independent sample *t*-test was used for comparing continuous variables. Categorical variables were compared between the two groups using chi-square test, and mean values of multiple groups were compared using ANOVA. The association between *VDR* polymorphism and T2DM was analyzed by conditional logistic regression. Hardy-Weinberg equilibrium (HWE) was assessed using the chi-square test based on observed and expected frequencies. The genetic risk score (GRS) was applied to confirm the additive genetic effect of the two SNPs on the risk of developing T2DM. Gene-environment interactions associated with T2DM were assessed using multifactor dimensionality reduction (MDR, version X, University of Virginia, USA). All the two-tailed statistical significances were defined at $P < 0.05$.

Results of the comparisons among baseline characteristics and clinical characteristics of the subjects between the two groups are presented in Table 1. Subjects in the T2DM group had higher levels of BMI, WHR, TG, family history of diabetes, and

Table 1. Characteristics of Patients with T2DM and Control Subjects

Characteristics	T2DM (*n* = 287)	Control (*n* = 287)	*P* Value[a]
Age (years)	59.23 ± 12.32	59.23 ± 12.30	0.989
Gender, *n* (%)			1.000
Male	113 (39.4)	113 (39.4)	
Female	174 (60.6)	174 (60.6)	
BMI (kg/m^2)	26.45 ± 3.75	25.58 ± 3.30	0.004**
WHR	0.92 ± 0.65	0.90 ± 0.66	< 0.001**
HDL-C (mmol/L)	1.29 ± 0.28	1.40 ± 0.31	< 0.001**
TC (mmol/L)	4.74 ± 1.12	4.71 ± 0.98	0.896
TG (mmol/L)[b]	1.76 (1.07, 2.77)	1.25 (0.83, 2.02)	< 0.001**
25(OH)D (ng/mL)[b]	17.97 (14.84, 32.23)	19.25 (15.08, 44.75)	0.032*
Physical activity, *n* (%)[c]			0.010*
Low	137 (47.8)	103 (35.9)	
Moderate	54 (18.8)	57 (19.9)	
High	96 (33.4)	127 (44.2)	
Family history of diabetes, *n* (%)			0.010*
Yes	66 (23.0)	42 (14.6)	
No	221 (77.0)	245 (85.4)	
Hypertension, *n* (%)			0.001**
Yes	183 (63.8)	145 (50.5)	
No	104 (36.2)	142 (49.5)	

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; WHR: waist:hip ratio; HDL-C: high-density lipoprotein cholesterol; TG: total triglycerides; TC: total cholesterol; [a]χ^2 test was used for categorical variables, and Student's *t*-test was used for continuous variables; [b]Variables were natural log-transformed before statistical analysis; [c]Physical activity was divided into three categories (low, moderate, and high) according to the International Physical Activity Questionnaire; *$P < 0.05$, significant value compared with patients with T2DM; **$P < 0.01$, significant value compared with patients with T2DM.

hypertension than control subjects (P < 0.05). Compared with control subjects, patients with T2DM had lower concentrations of 25(OH)D (ng/mL), HDL-C (mmol/L), and TG (mmol/L) (P < 0.05).

None of the genotype frequencies were found to deviate from HWE in both groups. Table 2 shows the genotype and allele distributions of the two *VDR* gene SNPs (rs2189480 and rs3847987) in patients with T2DM and control subjects. For rs2189480, carriers with the CC genotype showed a lower risk of developing T2DM than the AA genotype carriers (adjusted P = 0.001). Under the recessive genetic model (CC/AC+AA), the association between rs2189480 and the decreased risk of developing T2DM was significant (adjusted P = 0.001). However, under the dominant genetic model (AC+CC/AA), no significant association was found (adjusted P = 0.136). Compared with allele A, allele C may have an association with a lower risk of developing T2DM (P < 0.003). For rs3847987, individuals who carried the AA and CA+AA genotypes were at a higher risk of developing T2DM than individuals with the CC genotype (adjusted P values = 0.084 and 0.029, respectively). Allele A was more frequent in patients than in controls when allele C was taken as the reference, which suggested that allele A was a risk factor for T2DM (P = 0.032).

To our knowledge, this is an advanced study on the rural population of China evaluating the association between the novel polymorphic sites (rs2189480 and rs3847987) of the *VDR* gene and the risk of developing T2DM. Our results are consistent with those of several studies[6], which have reported that vitamin D levels are associated with insulin resistance, immunoregulation, and cell dysfunction related to T2DM. Several polymorphisms of the *VDR* gene such as ApaI, BsmI, TaqI, and FokI SNPs have been widely reported to be associated with both alterations in circulating activated vitamin D levels and in vitro measures of gene expression[7]. However, whether there are other SNPs involved in the process of T2DM has not yet been completely elucidated. The novel locus rs2189480 is one of the *VDR* SNPs located in intron and consists of an A-C change. The majority of previous studies on rs2189480 have focused on the susceptibility of bone-related and other diseases[8]. This is a novel field for us to investigate the association between this locus and T2DM. The SNP rs3847987 is located at the 3' untranslated region (UTR) of the *VDR* gene, which is known to be involved in the regulation of gene expression, especially through the regulation of mRNA stability. To our knowledge, this research is the first epidemiological study to investigate the relationship between *VDR* rs3847987 and T2DM. A related study reported that variants of rs3847987 may confer a genetic protection from type 1 diabetes[9]. The association between *VDR* polymorphisms and T2DM remains inconclusive, with some studies reporting an affirmative conclusion, whereas others failing to demonstrate this hypothesis[10]. We assumed that the divergence could be caused due to the heterogeneity of the studied populations and, primarily, the small sample sizes. Therefore, further extensive and convincing studies are required to elucidate and support our findings.

We adopted the GRS to evaluate the combined effect of the allelic variance of the two SNPs on the prevalence of T2DM. The risks effects of T2DM for the first grade, second grade, and third grade (3 and 4 risk alleles) were 3.08-, 3.57-, and 4.11-fold compared to that of the reference grade 0 (0 risk allele), respectively (Supplementary Table S1, available in www.besjournal.com). Participants with higher risk alleles of the two variants had an increased likelihood of suffering from T2DM compared with those lacking the risk alleles.

Univariate logistic regression analysis of the association between environmental and individual risk factors and T2DM revealed that low physical activity, family history of T2DM, WHR, TG, and HDL-C levels exhibited a risk toward T2DM development (P < 0.05). We next determined the gene-environment interaction by selecting the two SNPs and these environmental factors into the MDR analysis software. Based on the testing balance accuracy and the minimal prediction error, the interaction model comprising rs2189480, rs3847987, and physical activity was considered to be the best model with a statistical significance of P < 0.001 (Table 3). Furthermore, individuals with allele A of rs2189480 and rs3847987 who were engaged in low physical activity were categorized as the high-risk group, and the others were defined as the low-risk group. The prevalence of T2DM in the high-risk group was significantly higher than that in the low-risk group [*OR* and 95% *CI* = 2.53 (1.81-3.54), P < 0.001].

T2DM is a typical metabolic disorder with a complex, multifactorial, and polygenic etiology. Both environmental and genetic factors such as lifestyle, family history, and gene mutations may play a role in the pathogenesis of T2DM. These inherent determinants interact with external factors to produce

Table 2. Genotype and Allele Frequency of the Two *VDR* Gene Polymorphisms in Patients with T2DM and Control Subjects

VDR	T2DM (*n* = 287)	Control (*n* = 287)	*OR* (95% *CI*)	*P* Value	Adjusted *OR* (95% *CI*)	*P* Value[c]
rs2189480						
AA	131 (45.64)	115 (40.07)	1.000			
AC	131 (45.64)	115 (40.07)	1.000 (0.698, 1.434)	1.000	0.939 (0.636, 1.387)	0.754
CC	25 (8.72)	57 (19.86)	0.385 (0.223, 0.664)	0.001	0.375 (0.210, 0.672)	0.001*
AC+CC/AA[a]	156 (54.36):131 (45.64)	172 (59.93):115 (40.07)	0.795 (0.569, 1.109)	0.177	1.302 (0.917, 1.901)	0.136
CC/AC+AA[b]	25 (8.72):262 (91.28)	57 (19.86):230 (80.14)	0.392 (0.234, 0.658)	< 0.001	0.391 (0.226, 0.675)	0.001*
A	393 (68.47)	345 (60.10)	1.000			
C	181 (31.53)	229 (39.90)	0.431 (0.339, 0.547)	< 0.001*		
rs3847987						
CC	171 (59.58)	199 (69.34)	1.000			
CA	98 (34.15)	80 (27.87)	1.399 (0.926, 1.986)	0.060	1.376 (0.935,2.025)	0.105
AA	18 (6.27)	8 (2.79)	2.770 (1.133, 6.773)	0.026	2.675 (1.008,7.098)	0.048*
CA+AA/CC[a]	116 (40.42):171 (59.58)	88 (30.66):199 (69.34)	1.497 (1.067, 2.102)	0.020	1.511 (1.043,2.189)	0.029*
AA/CA+CC[b]	18 (6.27):269 (93.73)	8 (2.79):279 (97.21)	2.429 (1.007, 5.856)	0.048	2.566 (1.014,6.493)	0.047*
C	440 (76.66)	478 (83.28)	1.000			
A	134 (23.34)	96 (16.72)	1.373 (1.036, 1.819)	0.032*		

Note. Data are represented as *n* (%); [a]Analyzed under the dominant model; [b]Analyzed under the recessive model; [c]Adjusted for age, high-fat diet, HDL-C, physical activity, hypertension, BMI, and family history of T2DM; *$P < 0.05$, significant value compared with patients with T2DM; $P < 0.01$, significant value compared with patients with T2DM.

Table 3. Summary Table of Best Gene-environment Interaction Models Using MDR

Combination	Bal.Acc.CV Raining	Bal.Acc.CV Testing	CVC	χ^2	*P* Value	*OR* (95% *CI*)
X3	0.56	0.55	10/10	8.80	0.003	1.66 (1.19, 2.32)
X1 X3	0.59	0.55	6/10	16.94	< 0.001	2.01 (1.44, 2.81)
X1 X2 X3	0.61	0.59	10/10	29.63	< 0.001	2.53 (1.81, 3.54)

Note. X1: rs2189480; X2: rs3847987; X3: physical activity; CVC: cross-validation consistency; Bal.Acc.: balance accuracy.

the final pathogenic effect[11]. Thus, it makes sense to perform such analysis for managing the early prevention of T2DM.

In summary, this case-control study conducted in the Henan rural population identified two novel polymorphic loci (rs2189480 and rs3847987) of the *VDR* gene that are associated with the risk of developing T2DM. We also found that these two polymorphic loci can interact with the risk factor of low physical activity to increase the risk of T2DM development.

All the authors declare no conflict of interest. These authors contributed equally to this work.

[#]Correspondence should be addressed to LI Wen Jie, professor, Tel: 86-371-67781305, Fax: 86-371-67781868, E-mail: Lwj@zzu.edu.cn

Biographical note of the first author: HAN Han, born in 1992, master, majoring in nutrition and food hygiene.

Received: September 6, 2018;
Accepted: December 11, 2018

REFERENCES

1. Muoio DM, Newgard CB. Mechanisms of disease: Molecular and metabolic mechanisms of insulin resistance and beta-cell failure in type 2 diabetes. Nat Rev Mol Cell Biol, 2008; 9, 193-205.
2. Mirhosseini N, Vatanparast H, Mazidi M, et al. The Effect of Improved Serum 25-Hydroxyvitamin D Status on Glycemic Control in Diabetic Patients: A Meta-Analysis. J Clin Endocrinol Metab, 2017; 102, 3097-110.
3. Mackawy AM, Badawi ME. Association of vitamin D and vitamin D receptor gene polymorphisms with chronic

inflammation, insulin resistance and metabolic syndrome components in type 2 diabetic Egyptian patients. Meta Gene, 2014; 2, 540-56.
4. Liu X, Wang L, Wang P, et al. The Dynamics of Type 2 Diabetes Mellitus Prevalence and Management Rates among Rural Population in Henan Province, China. J Diabetes Res, 2017; 2017, 9092759.
5. Retnasabapathy A. Nutrition recommendations and interventions for diabetes: a position statement of the American Diabetes Association. Wiley, 2008; S61-78p.
6. Palomer X, Gonzálezclemente JM, Blancovaca F, et al. Role of vitamin D in the pathogenesis of type 2 diabetes mellitus. Diabetes Obes Metab, 2010; 10, 185-97.
7. Bid HK, Konwar R, Aggarwal CG, et al. Vitamin D receptor (FokI, BsmI and TaqI) gene polymorphisms and type 2 diabetes mellitus: a North Indian study. Indian J Med Sci, 2009; 63, 187-94.
8. Kiel DP, Demissie S, Dupuis J, et al. Genome-wide association with bone mass and geometry in the Framingham Heart Study. Bmc Medical Genet, 2007; 8, S14.
9. Ramos-Lopez E, Jansen T, Ivaskevicius V, et al. Protection from type 1 diabetes by vitamin D receptor haplotypes. Ann N Y Acad Sci, 2010; 1079, 327-34.
10. Li L, Wu B, Liu JY, et al. Vitamin D receptor gene polymorphisms and type 2 diabetes: a meta-analysis. Arch Med Res, 2013; 44, 235-41.
11. Jyothi KU, Reddy BM. Gene-gene and gene-environment interactions in the etiology of type 2 diabetes mellitus in the population of Hyderabad, India. Meta Gene, 2015; 5, 9-20.

Supplementary Table S1. Association between the GRS of the Two SNPs and T2DM

Grade[a]	T2DM (*n* = 287)	Control (*n* = 287)	*OR* (95% *CI*)	*P* Value	Adjusted *OR* (95% *CI*)	Adjusted *P* Value
0	18 (6.3)	51 (17.8)	1.00	—	—	—
1	96 (33.4)	96 (33.4)	2.83 (1.54, 5.20)	0.001	3.08 (1.64, 5.79)	< 0.001**
2	69 (24.0)	59 (20.6)	3.32 (1.75, 6.28)	< 0.001	3.57 (1.84, 6.95)	< 0.001**
3+4	104 (36.2)	81 (28.2)	3.64 (1.98, 6.70)	< 0.001	4.11 (2.17, 7.79)	< 0.001**

Note. [a]Number of risk alleles from rs2189480 and rs3847987. The genetic risk score (GRS) was graded as follows: Grade 0, GRS 0; Grade 1, GRS 1; Grade 2, GRS 2; Grade 3+4, GRS 3 and 4. **$P < 0.01$, significant value compared with patients with T2DM. Adjusted for age, high-fat diet, BMI, HDL-C, physical activity, hypertension, and family history of T2DM.