Letter to the Editor

Association of *VDR* Gene Variants with Hyperglycemia in Henan Rural Population*

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The term hyperglycemia was used to defined an abnormal status in which blood glucose increases and deviates from the normal fasting glucose (NFG), which contains the following statuses: impaired fasting glucose (IFG), impaired glucose tolerance (IGT), and diabetes mellitus (DM). According to the International Diabetes Federation Diabetes Atlas, nearly 537 million adults were suffering from diabetes in 2021 and the number is expected to reach 783 million by 2045[1]. T2DM causes serious complications and impairs the life quality of patients and brings economic burdens to the families and society. IFG and IGT are forms of prediabetes, and the cumulative incidence of T2DM progression five years after diagnosis is estimated to be 50%, it also indicates an already heightened risk of cardiovascular disease[2]. Therefore, the prevention and control of hyperglycemia has become a global public health problem that needs to be solved urgently. Hyperglycemia is considered attributed to be induced by a combination of genetic and environmental impacts.

Epidemiological studies have found that patients with hyperglycemia have lower serum $25(OH)D_3$ levels, and individuals with vitamin D deficiency have a higher risk of developing hyperglycemia[3]. Vitamin D receptor (VDR) gene is widely distributed in various tissues and cells within human body, while its protein expression is detected in more than 30 types of cells; furthermore, it can also bind to $1,25(OH)_2D_3$ to exert its physiological effects *in vivo*. Numerous studies exploring the association of VDR gene mutations and hyperglycemia mainly focused on *ApaI*, *FokI*, *BsmI,* and *TaqI* genes[4]. However, few data are available on the association of *VDR* gene (rs2189480) with the risk of hyperglycemia. Thus, our study was conducted to investigate the contribution of *VDR* gene (rs2189480) polymorphisms on hyperglycemia among rural population in Henan province.

We selected Houzhai in Zhengzhou and Wuzhi in Jiaozuo of Henan Province in China as the study locations. Subjects were age 18 years and above, with kidney disease or vitamin D supplementation were excluded. In total, 1,898 subjects (852 males and 1,046 females) were recruited from June to July in 2013. The study was approved by Medical Ethics Committee of Zhengzhou University ([2015] MEC (S128)), and informed consent forms were obtained from all subjects involved.

All participants completed questionnaire surveys on personal information contained basic issues, lifestyle and family history of T2DM (FAMTD). Measurement of body weight, height and blood pressure (hypertension: systolic blood pressure ≥ 140 mmHg and/or diastolic blood pressure ≥ 90 mmHg, or taking antihypertensive medication) were made and body mass index (BMI) was calculated (BMI = body weight/height square, normal range: $18.5 \le BMI < 24.0$ kg/m^2). Blood were collected after participants fasted for at least eight hours, fasting blood glucose (FBG) and lipid profiles were detected using automatic biochemical analyzer (KHB, Shanghai, China), and FBG was classified according to the American Diabetes Association standards: $3.9 \le FBG < 5.6$ mmol/L was known as NFG; $5.6 \le FBG < 7.0$ mmol/L was considered as IFG; T2DM was identified as FBG ≥ 7.0 mmol/L or with antidiabetic drugs[5]; the normal range of total cholesterol (TC) and triglyceride (TG) were less than 5.2 and 1.7 mmol/L, respectively; and derived indicators: homeostasis model assessment of insulin resistance

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(HOMA-IR) = (FBG × INS)/22.5, homeostasis model assessment of β-cell function (HOMA-β) = (INS × 20)/(FBG−3.5). Serum $25(OH)D_3$ and $1,25(OH)_2D_3$ were measured by corresponding (Sangon Biotech, Shanghai, China). Genomic DNA was extracted from peripheral blood using standard procedures (DNA blood kit, Bioteke, Beijing, China). Genotyping was performed using TaqMan probe assays and employing an Applied Biosystems (ABI, 7500 FAST Real-time PCR system, Foster City, USA) platform.

Normality distributed variables were shown as mean ± standard deviation (SD), and the differences between groups were assessed using one-way analysis of variance (ANOVA) with Bonferroni post-hoc test; non-normally distributed variables showed median (P_{25}, P_{75}), the one-way ANOVA was used for comparison between groups if the normally distribution was satisfied after log transform, otherwise the Kruskal Wallis H test was used. Categorical variables were reported as number (percentages) and compared using Chi-square (χ^2) test. Logistic regression analysis was used to evaluate the associations between genotypes and hyperglycemia. Genotype distributions were tested for Hardy-Weinberg Equilibrium (HWE), and the differences in genotype distribution were tested using the Chi-square test. The association between rs2189480 and the clinical and metabolic characteristics was assessed using general liner model with age, gender, BMI, drinking, smoking, physical activity and FAMTD as covariates. Gene-environmental factors interactions were assessed by generalized multifactor dimensionality reduction (GMDR, version 0.9, University of Virginia, USA). All statistical analyses were performed using SAS version 9.4 (SAS Institute, Cary, NC, USA) or SPSS version 26.0 (SPSS, Inc. Chicago, IL, USA) unless otherwise stated. Results with $P < 0.05$ (two-side) indicated a significant difference.

Of the 1,898 subjects analyzed in the study, 1,281 were NFG, 247 were IFG, and 370 were T2DM (Supplementary Table S1, available in www.besjournal.com). Consistent with previous findings[6], we found that participants with IFG and T2DM tended to be with higher age, BMI, TC, TG, HOMA-IR, lower HOMA-β, more FAMTD and less physically activity than NFG. Besides, individuals with IFG had higher low-density lipoprotein cholesterol (LDL-C) and lower high-density lipoprotein cholesterol (HDL-C) compared with NFG; individuals with T2DM showed lower $1,25(OH)_2D_3$ compared with NFG. $25(OH)D_3$ as the main circulating metabolite of vitamin D, is an important clinical index for measuring the clinical vitamin D nutrition level in human body, while $1,25(OH)_2D_3$ is the main active form of vitamin D, which combine with VDR and then exert a series of biological effects. A meta-analysis by Mohammadi[7] suggested that serum vitamin D levels were inversely correlated with T2DM risk; however, no such association was found in prediabetes. Consistent with Mohammadi[7], our study found that the level of serum $1,25(OH)_2D_3$ in T2DM patients was significantly lower than NFG. Intriguingly, the serum $25(OH)D_3$ level in IFG and T2DM subjects were numerically lower than NFG, but no remarkable difference had been observed yet. As a regulator of the vitamin D metabolic pathway, VDR could form a complex with active vitamin D and mediate its function in the nucleus. Hence, its expression or gene polymorphisms may not be significantly related to the concentration of $25(OH)D_3$ in serum.

In the present study, one polymorphic locus within the *VDR* gene, as well as its susceptibility to hyperglycemia in a Henan rural population was analyzed. The frequencies of the rs2189480 genotype were reported in Table 1. It is statistically significant for the distribution ($P = 0.037$), certainly, its genotype frequency did not deviate from the HWE ($\chi^2 = 2.518$, $P = 0.113$). Logistic regression analysis showed that CC carriers of rs2189480 were lower subjected to T2DM compared with AA carriers ($P = 0.010$), adjusted *OR* (95% *CI*) was 0.57 (0.37, 0.87); and dominant model showed similar result ($P = 0.011$), the adjusted *OR* (95% *CI*) was 1.70 (1.13, 2.56). Compared with NFG, the allele A has the highest proportion in T2DM population, indicating that allele A was a risk gene for T2DM, while the allele C was a protective gene ($P = 0.018$), adjusted *OR* (95% *CI*) was 0.81 (0.68, 0.97), suggesting a protective role of the allele in T2DM of the Henan rural population.

Yang et al.[8] found that *VDR* gene polymorphism could control gene expression by affecting mRNA localization, stability and translation and then influence the function and expression of protein, which may affect the effect of vitamin D. Subgroup analyses were performed to test whether the rs2189480 was associated with biochemical indicators and vitamin D concentrations. In the subcohort including NFG and IFG subjects, neither of the biochemical data was associated with rs2189480 (Supplementary Table S2, available in www.besjournal.com). Then, we excluded 247 IFG subjects from the statistical analysis, in the remaining NFG cohort (Table 2), there were significant associations

between rs2189480 and TG, $1,25(OH)_2D_3$ concentration in NFG subjects (P = 0.023; P = 0.022), the association still existed after adjustment for covariates (P = 0.024 for TG); CA/CC carriers showed a trend to association with higher $1,25(OH)_2D_3$ and lower TG levels compared with AA carriers.

Table 1. Genotype distribution of rs2189480 and its association with hyperglycemia

rs2189480	NFG (n = 1,281)	IFG (n = 247)	T2DM (n = 370)	χ^2	P	*OR* (95% *CI*)[a1]	P^a	*OR* (95% *CI*)[a2]	P^a
Additive model, *n* (%)				10.188	0.037				
AA	533 (42.03)	101 (41.22)	170 (46.20)			1 (Ref.)		1 (Ref.)	
CA	558 (44.01	104 (42.45)	166 (45.11)			0.98 (0.72, 1.34)	0.920	0.94 (0.72, 1.21)	0.612
CC	177 (13.96)	40 (16.33)	32 (8.70)			1.21 (0.80, 1.84)	0.364	0.57 (0.37, 0.87)	0.010
Recessive model				2.283	0.319				
AA/CA+CC	533:735	101:144	170:198			0.96 (0.72, 1.28)	0.790	1.18 (0.93, 1.51)	0.183
Dominant model				9.220	0.010				
AA+CA/CC	1,091:177	205:40	336:32			0.82 (0.56, 1.20)	0.308	1.70 (1.13, 2.56)	0.011
Allele frequency, *n* (%)				6.860	0.032				
A	1,624 (64.04)	306 (62.45)	506 (68.75)			1 (Ref.)		1 (Ref.)	
C	912 (35.96)	184 (37.55)	230 (31.25)			1.07 (0.88, 1.31)	0.503	0.81 (0.68, 0.97)	0.018

Note. [a]: Adjusted for age, gender, BMI, drinking, smoking, physical activity, and FAMTD; [1]: IFG *vs*. NFG; [2]: T2DM *vs.* NFG. NFG, normal fasting blood glucose; IFG, impaired fasting blood glucose; T2DM, type 2 diabetes mellitus; *OR*, odds ratio.

Table 2. Quantitative metabolic traits in NFG subjects stratified according to genotype

SNP genotype	rs2189480			P	P^a
	AA	CA	CC		
n (%)	533 (42.03)	558 (44.01)	177 (13.96)		
BMI (kg/m^2)	24.78 ± 3.64	24.86 ± 3.72	24.38 ± 3.28	0.496	
FBG (mmol/L)	4.73 ± 0.45	4.76 ± 0.45	4.76 ± 0.42	0.745	0.778
INS (mIU/L)[b]	10.77 (8.05, 13.78)	10.72 (8.03, 13.52)	10.35 (7.60, 13.97)	0.919	0.690
TC (mmol/L)	4.50 ± 0.98	4.43 ± 1.01	4.36 ± 0.93	0.254	0.264
TG (mmol/L)[b]	1.34 (0.86, 1.98)	1.18 (0.78, 1.90)	1.11 (0.83, 1.70)	0.023	0.024
LDL-C (mmol/L)	2.56 ± 0.78	2.50 ± 0.79	2.49 ± 0.71	0.413	0.397
HDL-C (mmol/L)	1.24 ± 0.29	1.25 ± 0.32	1.29 ± 0.33	0.172	0.171
HOMA-IR[b]	2.28 (1.73, 2.97)	2.29 (1.66, 2.95)	2.24 (1.59, 3.06)	0.877	0.673
HOMA-β[b]	177.83 (127.22, 269.63)	169.45 (121.68, 256.73)	171.83 (119.06, 247.84)	0.891	0.834
$25(OH)D_3$ (ng/mL)[c]	19.49 (15.40, 28.58)	17.97 (14.92, 25.53)	19.70 (14.58, 29.64)	0.377	
$1,25(OH)_2D_3$ (ng/mL)[c]	22.13 (14.25, 39.56)	23.66 (17.81, 58.30)	22.78 (14.69, 49.53)	0.022	

Note. Data were given as means ± SD for normally distributed variables (BMI, FBG, TC, LDL-C, HDL-C), median (P_{25}, P_{75}) for non-normally distributed variables [INS, TG, HOMA-IR, HOMA-β, $25(OH)D_3$, $1,25(OH)_2D_3$], *n* (%) for categorical variables. [a]: Adjusted for age, gender, BMI, drinking, smoking, physical activity, and FAMTD. P value for general liner model. [b]: Normally distributed variables after log transform; [c]: Kruskal Wallis H test. SNP, single nucleotide polymorphism; BMI, body mass index; FBG, fasting blood glucose; INS, fasting serum insulin; TC, total cholesterol; TG, triglyceride; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; HOMA-IR, homeostasis model assessment of insulin resistance; HOMA-β, homeostasis model assessment of β-cell function.

Rs2189480 and environmental factors were analyzed using GMDR software to explore the gene-environment interactions on the probability of hyperglycemia. As presented in Supplementary Table S3 (available in www.besjournal.com), the results showed meaningful models involving rs2189480 and BMI/hypertension/TC/TG (P = 0.001); it indicated that there were potential interactions between rs2189480 and these environmental factors to influence hyperglycemia risk. Next, logistic regression were used to further analyze the potential factors. Table 3 demonstrated that the interaction between rs2189480 and BMI was not found after adjusting for covariates in logistic regression analysis. Nonetheless, it showed that CA+AA genotypes combined with hypertension had a higher risk of hyperglycemia (*OR* = 1.90, 95% *CI* 1.16, 3.12, P = 0.011); similarly, the combination of CA+AA genotypes with abnormal TC levels had the same results (*OR* = 1.74, 95% *CI* 1.17, 2.58, P = 0.006). For TG, except for normal TG combined CC genotype, the risk of hyperglycemia increased under other combinations ($P < 0.05$). Previous studies have shown that hypertension and dyslipidemia were significantly associated with the occurrence of diabetes[9,10]. Our results revealed that CA+AA carriers of rs2189480 with hypertension or abnormal TC/TG levels exhibit a higher risk of hyperglycemia. To summarize, daily monitoring and control of blood lipid, blood pressure may greatly reduce the risk of hyperglycemia.

Some limitations have existed in our study. First, there was a big difference in the sample size of the subgroups. Besides, this study only focused on one gene, and did not pay attention to other genes that may affect vitamin D metabolism. Nevertheless, our study also has some strengths. First, we completed the collection of anthropometric indicators and biological samples from June to July of the same year, avoiding the impact. Second, we only selected the Han population in Henan rural for the study to avoid the influence of genetic background of different ethnic groups and regions. Last, we performed gene-environment interaction analyses to explore whether and how they affect hyperglycemia risk.

In conclusion, our study found that the CA+AA genotypes of rs2189480 of the *VDR* gene may be the risk factor of hyperglycemia. Interactions between rs2189480 and hypertension/TC/TG could affect the

Table 3. Gene-environment interaction influencing hyperglycemia

Environment factors		Rs2189480	Case/control	*OR* (95% *CI*)	P^a
BMI	Normal	CC	18/76	1 (Ref.)	
		CA+AA	130/433	1.20 (0.68, 2.12)	0.522
	Abnormal	CC	54/100	1.22 (0.62, 2.38)	0.561
		CA+AA	404/654	1.48 (0.82, 2.66)	0.195
Hypertention	No	CC	24/103	1 (Ref.)	
		CA+AA	241/693	1.40 (0.86, 2.28)	0.172
	Yes	CC	48/73	1.69 (0.93, 3.09)	0.088
		CA+AA	294/395	1.90 (1.16, 3.12)	0.011
TC	Normal	CC	51/144	1 (Ref.)	
		CA+AA	365/854	1.18 (0.82, 1.69)	0.368
	Abnormal	CC	20/33	1.33 (0.68, 2.62)	0.406
		CA+AA	176/237	1.74 (1.17, 2.58)	0.006
TG	Normal	CC	27/131	1 (Ref.)	
		CA+AA	269/732	1.86 (1.18, 2.95)	0.008
	Abnormal	CC	44/44	4.40 (2.37, 8.15)	< 0.001
		CA+AA	266/354	3.25 (2.04, 5.19)	< 0.001

Note. [a]: Adjusted for age, gender, BMI, drinking, smoking, physical activity, and FAMTD. BMI: body mass index; TC, total cholesterol; TG, triglyceride; *OR*, odds ratio; Case: hyperglycemia; Control: normal fasting glucose.

risk of hyperglycemia. However, this result is only for the population in rural areas of Henan, and it is necessary to verify our results in multi-center, large-sample trials.

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