GC gene polymorphisms found with type 2 diabetes and low vitamin D status among rural Chinese in Henan province

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Mingming Feng MD, Dongdong Zhang MD, Yiming Liu MD, Tong Zhao MD, Haoyue Huang MD, Luoya Zhang MD, Xing Li PhD, Wenjie Li PhD

Department of Nutrition and Food Hygiene, College of Public Health, Zhengzhou University, Zhengzhou, Henan, China

Authors' email addresses and contributions:
Mingming Feng: mingmingfeng429@163.com
Contribution: conceived the study question, and contributed to the study design, supervision of data collection, data analysis and interpretation, and writing the manuscript.

Dongdong Zhang: dongdongzhang23@163.com
Contribution: undertook data collection and data analysis, and contributed to data interpretation.

Yiming Liu: yimingliu610@163.com
Contribution: undertook data collection and data analysis, and contributed to data interpretation.

Tong Zhao: 15588587191@163.com
Contribution: undertook data analysis and interpretation.

Haoyue Huang: haoyuehhy@163.com
Contribution: undertook data analysis and interpretation.

Luoya Zhang: z623102334@163.com
Contribution: undertook data analysis and interpretation.

Xing Li: lixing530@zzu.edu.cn Contribution: undertook the revisions and critique.

Wenjie Li: lwj@zzu.edu.cn
Contribution: conceived the study question; contributed to the study design.

Corresponding Author: Dr Wenjie Li, Department of Nutrition and Food Hygiene, College of Public Health, Zhengzhou University, 100 Kexue Avenue, Zhongyuan District, Zhengzhou, Henan, China. Tel: +86 371 6778 1305. Fax: +86 371 6778 1868. Email: lwj@zzu.edu.cn

ABSTRACT

Background and Objectives: Accumulating evidence suggests that vitamin D may be involved in the pathogenesis of type 2 diabetes (T2D). Group specific component (*GC*) gene is the most important transporter of vitamin D and plays a regulatory role in vitamin D metabolism. We aimed to evaluate the association of *GC* gene polymorphisms with T2D susceptibility and vitamin D status in the Chinese rural population. **Methods and Study Design:** A total of 1372 subjects were eligible in this cross-sectional study. Three SNPs of the GC gene (rs7041, rs4588, and rs2282679) were genotyped by TaqMan probe assays. Logistic regression and Kruskal-Wallis one-way analysis were performed to determine the possible risk genotype for T2D and vitamin D metabolite concentrations, respectively. **Results:** The serum 25-hydroxyvitamin D_3 [25(OH)D_3] and vitamin D binding protein (DBP) concentrations were significantly lower in the T2D group than the non-T2D group. GG genotype carriers of rs7041 (T>G) were more likely to have T2D compared with AA carriers (OR=2.00, 95% CI: 1.19-3.37). Variants of rs4588 (C>A) and rs2282679 (A>C) were associated with a lower risk of T2D under the dominant inheritance model (OR=0.65, 95% CI: 0.48-0.88; OR=0.66, 95% CI: 0.49-0.90, respectively). We further found that non-T2D subjects with AA genotype of rs4588 had significantly higher 25(OH)D3 concentrations than the CC genotype (p=0.022). In contrast, the T2D cases with the CC genotype of rs2282679 had lower DBP concentrations compared to the AA genotype (p=0.020). **Conclusions:** Our study indicates a potential role for GC gene polymorphisms in T2D susceptibility and vitamin D metabolite concentrations in the Chinese rural population.

Key Words: *GC* gene, polymorphism, type 2 diabetes, vitamin D binding protein, 25(OH)D_3

INTRODUCTION

Type 2 diabetes (T2D) is a complex metabolic disease which is characterized by insulin resistance, defective insulin secretion, or both of them.[1] The 9th edition of the International Diabetes Federation Diabetes Atlas predicts that the number of diabetic patients will increase to 578 million by 2030 and 700 million by 2045.[2] T2D can lead to serious complications such as cardiovascular disease, stroke, kidney failure and retinopathy.[3] T2D is a multifaceted disorder with several risk factors, including genetics, diet, and personal behaviors, of which genetics plays a critical role with an estimated heritability of 40% to 80%.[4]

As the main circulating vitamin D metabolites, serum 25-hydroxyvitamin D_3 [25(OH)D_3] is a clinical biomarker for judging the status of vitamin D. Vitamin D binding protein (DBP) is a protein encoded by *GC* gene, which is converted into various forms by combining with vitamin D and transported to various organs of the body.[5] *GC* is located on chromosome 4q13 and has 13 exons, encoding 474 amino acids. There are three common single nucleotide polymorphisms (SNPs) of the *GC* gene, which are rs7041 (T>G) and rs4588 (C>A) located in exon 11 and rs2282679 (A>C) located in intron 12.[6] DBP concentrations are susceptible to *GC* gene polymorphism, which affects the affinity of vitamin D and its metabolites, resulting in changes in the concentration of 25(OH)D_3.[7] Studies have demonstrated that *GC* gene was involved in circulating 25(OH)D concentration.[8,9]

Due to the biological function of the GC gene, numerous researches tend to explore diseases related to vitamin D concentrations with the polymorphism of the *GC* gene, such as cancer, coronary artery disease, Parkinson's disease and Alzheimer's disease.[10-12] In addition, related studies have also been conducted to investigate the relationship between *GC* SNPs and T2D susceptibility, but the existing findings are inconsistent. Wang, et al. and Rahman, et al. demonstrated that GC SNPs were associated with increased risk of T2D in Asians and Bangladesh, respectively.[13,14] Whereas, Ye et al and Malecki et al did not find the evidence for an association between *GC* gene SNPs and T2D susceptibility in French Caucasians and Poland.[15,16]

Thus, the objective of the current study was to investigate the associations of (1) *GC* SNPs and T2D risk in a cross-sectional study; (2) the vitamin D-related metabolite concentrations and T2D risk; (3) serum vitamin D-related metabolite concentrations and *GC* SNPs.

MATERIALS AND METHODS

Subjects

The subjects of this cross-sectional study were recruited by the method of cluster random sampling. Two towns, Wuzhi in Jiaozuo City and Houzhai in Zhengzhou City, were randomly selected from rural areas of Henan Province. Ultimately, a total of 1570 subjects were willing to participate and complete the examination in July to August 2013 and July to August 2015. The present analysis excluded individuals younger than 18 years of age (n=102), failing in DNA extraction (n=24) or vitamin D extreme values (n=72). Finally, we obtained a sample of 1372 participants. The detailed flow chart of the research object is shown in Figure 1. We collected data with basic information such as age, gender, physical activity, history of chronic diseases, culture and vitamin D supplement intake during face-to-face interviews. They then

completed a physical examination (height, weight, waist circumference and hip circumference), fasting blood glucose and blood lipid tests. According to the definition and standards of the World Health Organization (WHO),[17] T2D was defined as fasting plasma glucose ≥7.0 mmol/L and/or taking hypoglycemic drugs and/or having a self-reported history of T2D, we eventually identified 231 cases of T2D patients.

This study was approved by the Zhengzhou University Life Science Ethics Committee (Code: [2015] MEC (S128)). Written informed consent was obtained from all participants.

Biochemical measurements

Fast blood glucose and lipid were determined by the automatic biochemical analyzer (KHB360, Shanghai, China). Serum 25(OH)D_3 and DBP concentrations were detected by ELISA kits (Sangon Biotech, Shanghai, PR China). The absorbance of the microplate reader at 450 nm was measured. According to the Institute of Medicine (IOM),[18] we classified serum 25(OH)D_3 into three groups: vitamin D deficiency (VDD) (<20 ng/mL), vitamin D insufficient (VDI) (20-30 ng/mL), vitamin D sufficient (VDS) (≥30 ng/mL).

Genotyping

Genomic DNA was extracted from peripheral blood according to the standard procedures (DNA blood kit, Bioteke, Beijing, China). Based on the HapMap website and previous research, three SNPs of the GC gene (rs7041, rs4588 and rs2282679) were identified for further analysis. Genotyping was completed by TaqMan probe assays and employing an Applied Biosystems (ABI, 7500 FAST Real-time PCR system, Foster City, USA) platform. For genotyping quality control, we selected 10% random samples for duplicated analysis, and the concordance rate was 99.5%.

Sample size and statistical analyses

We used the previous study of GC variants and T2D reported by Fawzy19 to obtain a rough estimate of the odds ratio (OR=2.50) and considered the minor allele frequencies (MAF) of rs2282679 as the prevalence of risk factor (p=0.178). Based on this, we estimated a required sample size of 124 to detect an effect of similar magnitude at an α level of 0.05 with 90% statistical power.

Statistical analyses were performed using IBM SPSS 21.0 (SPSS, Chicago, IL, USA). Continuous variables conforming to normality were compared by Student's t-test for two groups, which were represented by means ± standard deviations (SD). Otherwise, Wilcoxon

rank sum test was used, and the data were expressed as medians (interquartile ranges). Categorical variables were assessed using chi-square test. We used goodness-of-fit χ^2 test to examine Hardy–Weinberg equilibrium. Linkage disequilibrium were determined using Haploview. Logistic regression models were performed to evaluate the relationship between the three SNPs of the *GC* gene and the risk of T2D. The results were corrected for multiple comparisons using false discovery rate (FDR). Kruskal-Wallis one-way analysis was performed to estimate the association between serum 25(OH)D_3 and DBP concentrations and *GC* SNPs. Multivariate ORs and 95% confidence intervals (CI) for T2D according to the divided serum vitamin D metabolite quartiles and clinical thresholds for 25(OH)D_3 were calculated using a logistic regression while adjusting for age, gender, married status, smoking status, drinking status, high-fat intake, physical activity, and family history of diabetes. We also conducted subgroup analysis based on age, gender, family history of diabetes, hypertension or not and vitamin D status to further identify the robustness of our results. A two-sided *p*-value <0.05 was considered to be statistically significant.

RESULTS

Characteristics of the subjects

A total of 231 individuals with T2D and 1141 non-T2D fulfilled the criteria and were included in the analysis. The T2D group showed higher concentrations of total cholesterol (TC), triglyceride (TG), low-density lipoprotein cholesterol (LDL-C) than the non-T2D group. Inversely, the high-density lipoprotein (HDL-C), serum 25(OH)D_3 and DBP concentrations of T2D patients were lower than that of the non-T2D group (p<0.05). There was a significant difference in the distribution of vitamin D status between the T2D and non-T2D groups (p=0.001). The percentage of T2D patients with VDD and VDI was 74.0%. No significant difference was found in the family history of diabetes between the two groups (p=0.403). Clinical characteristics of the T2D and the non-T2D group are presented in Table 1.

Association between GC polymorphisms and T2D

All genotype frequencies of the two groups were in Hardy–Weinberg equilibrium (p>0.05). The results of linkage disequilibrium between the three SNPs are shown in Supplementary Figure 1. Table 2 summarizes the genotype distribution of three SNPs between the T2D and non-T2D group. The three SNPs (rs7041/rs4588/rs2282679) were all significantly different in the distribution of the two groups (p=0.007, 0.020 and 0.022, respectively). Moreover, rs4588 (C>A) and rs2282679 (A>C) were associated with a lower risk of T2D under the dominant

inheritance model (OR=0.65, 95% CI: 0.48-0.88; OR=0.66, 95% CI: 0.49-0.90). Notably, for rs7041 (T>G) variant, carriers of the GG genotype were more likely to suffer from T2D compared with those carrying TT genotype (OR=2.00, 95% CI: 1.19-3.37).

To further explore the effect of other risk factors on the association of GC gene SNPs with T2D risk, subgroup analyses were performed based on age, gender, family history of diabetes, hypertension or not and vitamin D status (Table 3). Overall, the associations of SNPs and T2D were consistent in most of the subgroups. In addition, rs7041 polymorphism was associated with a higher risk of T2D in subjects with a family history of diabetes and without hypertension. It should be noted that rs4588 and rs2282679 were associated with a lower risk of T2D in female, subjects with age <60 years and no family history of diabetes, under the dominant inheritance model.

Vitamin D metabolite concentrations

We analyzed the distribution of vitamin D metabolites in the three SNP genotypes in T2D and non-T2D groups (Table 4). The rs4588 genotype was significantly associated with serum 25(OH)D_3 concentrations in the non-T2D group, and the AA genotype had a significantly higher serum 25(OH)D_3 concentrations than did those with the CC genotype (p=0.022). However, no significant association was found either between rs7041 and 25(OH)D_3 or between rs2282679 and 25(OH)D_3 in any of the groups. In addition, the rs2282679 genotype was associated with serum DBP concentrations in the T2D group, showing that the CA genotype was associated with lower DBP concentrations compared with the AA genotype (p=0.020). And rs7041 and rs4588 variants had no significant association with DBP in either the T2D or non-T2D groups.

The association of serum vitamin D metabolite concentrations and T2D risk was also investigated (Supplementary Table 1). For both serum 25(OH)D_3 and DBP, the risk of T2D in quartile 2 (Q2) was significantly higher than those in Q4 (OR=1.92, 95% CI: 1.20-3.06; OR=1.76, 95% CI: 1.12-2.76, respectively), after adjusting for age, gender, marry status, education, smoking status, drinking status, physical activity, family history of diabetes.

DISCUSSION

Vitamin D is related to the pathogenesis of T2D, and the *GC* gene, as the most important transporter of vitamin D, which plays a regulatory role in vitamin D metabolism. Exploring the relationship between *GC* SNPs and T2D as well as vitamin D may provide valuable information for the prediction, occurrence, and development of T2D. Our study indicates that

GC variants (rs7041, rs4588 and rs2282679) are associated with T2D risk in a Chinese rural population. It was also found that lower vitamin D concentrations were associated with a higher T2D risk. Furthermore, we found that rs4588 and rs2282679 genotypes were associated with vitamin D metabolite concentrations.

Several studies have investigated the association of *GC* gene polymorphisms and the risk of T2D with conflicting results.[13,15,16,20] Zhao et al demonstrated a significant difference between rs7041 polymorphism and T2D incidence.[21] Ye et al and Malecki et al did not find the evidence for an association between *GC* gene SNPs and T2D in French Caucasians and Poland.[15,16] Rahman et al and Hirai et al. identified that there was an association of *GC* SNPs and T2D in Bangladesh and Japan respectively.[13,20] In our study, we found that rs7041, rs4588 and rs2282679 polymorphisms within the *GC* gene were all significantly associated with risk of T2D in Chinese rural population. Moreover, our results revealed that the mutation of rs7041 was associated with a higher risk of T2D, while the mutations of rs2282679 and rs4588 were a protective factor for the progression of T2D. It has been reported that beta cells behave abnormally when islet fatty acid concentration increases. Therefore, we speculate that *GC* gene variants may alter fatty acid concentrations and thus affect the development of diabetes.[22] In addition, results of subgroup analysis revealed that the mutation of rs4588 and rs2282679 were associated with a lower risk of T2D in female, subjects younger than 60 years and without a family history of diabetes. To the best of our knowledge, gender, genetic factors and age have been considered as high risk factors for T2D. Male have a higher risk of T2D than female at lower age and body mass index.[23] Relevant reports indicated parental history of diabetes was related to a 2-6 times increase in diabetes risk.[24,25] Compared with younger ages, diabetes were more prevalent among older ages (60–79 years).[26,27] No significant difference was found among male, subjects over 60 years and with a family history of diabetes, which can be explained by the distinct effects of gender, age and family history of diabetes on T2D may blur the association between *GC* SNPs and T2D risk. In addition, we found that rs7041 was associated with an increased T2D risk in subjects without hypertension. In our study, most patients with hypertension have taken antihypertensive drugs such as calcium channel blockers, angiotensin-converting enzyme inhibitors, or angiotensin-receptor blockers, which may decrease the incidence of diabetes.[28] This could interfere with genetic effects on the progression of T2D among these patients.

Increasing evidences have suggested that vitamin D has a protective effect on T2D, and high 25(OH)D_3 concentrations in the body are associated with a lower risk of T2D.[29-31] As the main protein carrier of serum 25(OH)D_3, DBP is considered to be the main dominant factor of

25(OH)D_3 concentrations. Therefore, simultaneous measurement of DBP concentrations may reveal a causal pathway between 25(OH)D_3 and risk of T2D. Our study discovered that the concentrations of serum 25(OH)D_3 and DBP in the T2D group were significantly lower than in the non-T2D group, which is consistent with the results of other studies.[32-35] Additionally, we found that both lower concentrations of 25(OH)D_3 and DBP were associated with an increased risk of T2D compared with the highest quartile. The relationship between vitamin D and T2D may be explained by the following mechanisms. First, vitamin D can regulate insulin secretion by maintaining calcium pools balance *in vitro* and *in vivo*.[36] Second, vitamin D can reduce the production and release of inflammatory factors, inhibit low-grade inflammatory response, and increase the expression of insulin receptor genes in insulin resistance target organs (fat, skeletal muscle and liver), thereby improving the tissue response to insulin.[37] Third, vitamin D may suppress the secretion of parathyroid hormone in the body and the renal angiotensin aldosterone system, thereby improving insulin sensitivity.[38-40] In conclusion, vitamin D may affect the progression of T2D by regulating insulin secretion and insulin resistance.

Sedky et al. and Zhang et al. also explored the association between the GC SNPs (rs7041, rs4588 and rs2282679) and serum 25(OH)D_3 concentrations, but they did not find a significant difference.[11,41] Contrarily, Ahn et al. and Wang et al. in their studies found that rs2282679 variants were associated with lower serum 25(OH)D_3 concentrations in white Europeans.[8,9] In our study, rs4588 genotypes were significantly associated with serum 25(OH)D_3 concentrations in the non-T2D group and rs2282679 genotype was associated with serum DBP concentrations in the T2D group. The results of our study may suggest a role of GC polymorphisms in regulating vitamin D metabolite concentrations and T2D risk. Previous studies have shown that 1,25 dihydroxyvitamin D3 [1,25(OH)2D3], a metabolite of 25(OH)D_3, influences insulin secretion and insulin resistance.[42,43] The 1,25(OH)2D_3 could directly bind to vitamin D receptors (VDR) in pancreatic β cells, and stimulate the production of insulin by regulating gene transcription.[44] On the other hand, 1,25(OH)2D_3 could mediate the transcriptional activation of insulin receptor genes, increasing the number of insulin receptors on the surface of target cells, thus promoting insulin signaling and maintaining insulin sensitivity.[45] Therefore, we hypothesized that *GC* variants could affect serum DBP concentrations, leading to changes in the concentrations of 1,25(OH)2D_3, and thus playing a critical role in the occurrence and development of T2D.

In summary, our study suggests that the risk of T2D may be influenced by the *GC* gene polymorphisms and serum 25(OH)D_3 concentrations. Vitamin D plays a pivotal role in insulin

secretion and insulin sensitivity, and variations in the *GC* gene can affect the concentrations of vitamin D metabolites, which in turn affect the secretion of insulin, leading to disorders of glucose metabolism and even the occurrence of T2D. Our findings encourage further research to elucidate the mechanisms underlying T2D.

The advantage of the present study is that we found a significant association between rs2282679 variant and T2D risk in a Chinese rural population. But limitations should be noted. First, this study was conducted in July and August each year when serum $25(OH)D_3$ measured in summer may be higher due to sunlight and skin exposure. Second, given that our study included only Chinese rural population, the conclusions observed in our research may not be directly extrapolated to other races. Third, we only focused on the *GC* gene involved in vitamin D metabolism. It is not clear whether there are other genes or factors that interact with the *GC* gene to cause T2D.

Conclusion

GC gene polymorphisms (rs7041, rs4588 and rs2282679) are associated with T2D risk in a Chinese rural population. Lower concentrations of $25(OH)D_3$ and DBP were associated with a higher risk of T2D. Further, we found rs7041 and rs4588 were associated with $25(OH)D_3$ and DBP concentrations. These findings highlight the importance of genetic polymorphisms in T2D progression and suggest that a complex combination of vitamin D metabolites and *GC* SNPs may underlie T2D susceptibility. Genetic studies could screen for risk genotypes to complement traditional diabetes risk factors, so identifying high-risk patients earlier.

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CONFLICT OF INTEREST AND FUNDING DISCLOSURE

The authors have no relevant interests to declare.

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Table 1. Baseline characteristics of study participants

Characteristics	T2D (n=231)	Non-T2D (n=1141)	Statistics	*p* value
Gender			6.33	0.012
Male	96 (41.7%)	580 (50.8%)		
Female	134 (58.3%)	561 (49.2%)		
Age (years)			66.6	<0.001
⩽40	18 (7.8%)	354 (31.0%)		
40-60	90 (39.0%)	437 (38.3%)		
≥60	123 (53.3%)	350 (30.7%)		
TC (mmol/L)	4.61 (3.95, 5.51)	4.34 (3.68, 5.06)	12.4	0.001
TG (mmol/L)	1.69 (1.05, 2.67)	1.17 (0.76, 1.92)	40.5	<0.001
HDL-C (mmol/L)	1.27 (1.10, 1.47)	1.34 (1.15, 1.55)	8.88	0.003
LDL-C (mmol/L)	2.49 (1.97, 3.09)	2.32 (1.85, 2.87)	5.09	0.024
25(OH)D_3 (ng/mL)	18.4 (14.9, 32.4)	21.1 (15.2, 50.4)	7.01	0.008
DBP (ng/mL)	124 (99.2, 239)	155 (103, 399)	11.6	0.001
Family history of diabetes			1.82	0.403
Yes	53 (22.9%)	264 (23.1%)		
No	174 (75.3%)	886 (76.1%)		
Vitamin D status			13.6	0.001
VDD	129 (55.8%)	540 (47.3%)		
VDI	42 (18.2%)	160 (14.0%)		
VDS	60 (26.0%)	441 (38.7%)		

DBP: vitamin D binding protein; VDD: Vitamin D deficiency; VDI: vitamin D insufficient; VDS: vitamin D sufficient.
Data are expressed as the frequency and percentage (%) or medians (interquartile ranges), with the significance of differences between groups evaluated using the $\chi 2$ test, or Wilcoxon rank sum test, respectively.

Table 2. Effect of GC gene SNPs on T2D risk

SNPs	T2D	Non-T2D	χ^2	*p*	OR (95% CI)	*p*	*p-FDR*	*p-HWE*
rs7041			9.94	0.007				0.162
TT	114 (49.4%)	638 (55.9%)			Reference			
TG	90 (39.0%)	433 (38.0%)			1.12 (0.82, 1.55)	0.472	0.472	
GG	27 (11.7%)	70 (6.10%)			2.00 (1.19, 3.37)	0.010	0.030	
TG+GG					1.25 (0.93, 1.68)	0.147	0.221	
rs4588			7.78	0.020				0.763
CC	126 (54.6%)	510 (44.7%)			Reference			
CA	88 (38.1%)	514 (45.1%)			0.70 (0.51, 0.96)	0.028	0.028	
AA	17 (7.40%)	117 (10.3%)			0.46 (0.26, 0.84)	0.011	0.017	
CA+AA					0.65 (0.48, 0.88)	0.006	0.017	
rs2282679			7.61	0.022				0.584
AA	126 (54.6%)	510 (44.7%)			Reference			
CA	87 (37.7%)	513 (45.0%)			0.70 (0.51, 0.96)	0.029	0.029	
CC	18 (7.80%)	118 (10.3%)			0.51 (0.29, 0.91)	0.023	0.029	
CA+CC					0.66 (0.49, 0.90)	0.008	0.024	

SNP: single-nucleotide polymorphism; OR: odds ratio; CI: confidence interval.
Data are expressed as the frequency and percentage (%).
Adjusted for age, gender, smoking, drinking, exercise, marry status, occupation, culture, family history of diabetes and 25(OH)D3.

Table 3. Effect of GC gene SNPs on T2D risk stratified by subgroups

Variables	T2D	Non-T2D	rs7041		rs4588		rs2282679	
			OR (95% CI)	*p*	OR (95% CI)	*p*	OR (95% CI)	*p*
Age (years)								
<60	107 (7.80%)	780 (56.9%)	1.50 (0.96, 2.32)	0.073	0.57 (0.37, 0.89)	0.013	0.57 (0.37, 0.88)	0.012
≥60	124 (9.00%)	361 (26.3%)	1.13 (0.73, 1.75)	0.597	0.75 (0.48, 1.17)	0.205	0.78 (0.50, 1.22)	0.274
Gender								
Male	96 (7.00%)	580 (42.3%)	1.38 (0.87, 2.18)	0.173	0.69 (0.44, 1.10)	0.121	0.72 (0.45, 1.14)	0.162
Female	134 (9.80%)	561 (40.9%)	1.13 (0.75, 1.69)	0.558	0.63 (0.42, 0.94)	0.029	0.62 (0.41, 0.93)	0.022
Family history of diabetes								
Yes	53 (3.90%)	264 (19.2%)	2.07 (1.03, 4.16)	0.040	0.80 (0.40, 1.57)	0.512	0.87 (0.44, 1.70)	0.674
No	174 (12.7%)	869 (63.3%)	1.08 (0.76, 1.52)	0.667	0.65 (0.46, 0.92)	0.016	0.65 (0.46, 0.91)	0.013
Hypertension								
Yes	133 (9.70%)	450 (32.8%)	1.00 (0.67, 1.50)	0.993	0.63 (0.42, 0.97)	0.032	0.68 (0.46, 1.03)	0.053
No	94 (6.90%)	685 (49.9%)	1.62 (1.02, 2.56)	0.041	0.50 (0.31, 0.80)	0.004	0.46 (0.28, 0.74)	0.001
VDD+VDI								
Yes	171 (12.5%)	700 (51.0%)	1.40 (0.98, 1.98)	0.062	0.67 (0.47, 0.96)	0.027	0.69 (0.49, 0.99)	0.045
No	60 (4.40%)	441 (32.1%)	0.99 (0.55, 1.82)	0.995	0.54 (0.30, 0.97)	0.039	0.52 (0.28, 0.95)	0.035

SNP: single-nucleotide polymorphism; VDD: Vitamin D deficiency; VDI: vitamin D insufficient; OR: odds ratio; CI: confidence interval.
Data are expressed as the frequency and percentage (%).
Adjusted for age, gender, smoking, drinking, exercise, marry status, occupation, culture, family history of diabetes and 25(OH)D3 under the dominant inheritance model.

Table 4. Distribution of vitamin D metabolites under different genotypes

SNPs	$25(OH)D_3$ (ng/mL)			χ^2	*p* value	DBP (ng/mL)			χ^2	*p* value
rs7041	TT	TG	GG			TT	TG	GG		
T2D	18.5 (15.5, 37.5)	17.2 (14.4, 28.5)	20.1 (16.3, 37.1)	4.07	0.131	125 (99.2, 276)	117 (97.7, 183)	147 (105, 199)	2.29	0.318
non-T2D	21.1 (15.2, 52.3)	20.3 (14.9, 45.3)	26.0 (16.9, 50.5)	2.63	0.269	154 (102, 412)	153 (102, 353)	171 (110, 423)	1.52	0.467
rs4588	CC	CA	AA			CC	CA	AA		
T2D	19.1 (15.0, 38.3)	17.5 (14.6, 29.6)	17.5 (16.8, 23.1)	1.61	0.446	137 (102, 285)	112 (96.2, 198)	122 (100, 252)	5.40	0.067
non-T2D	23.7 (15.3, 51.5)	20.4 (14.7, 44.8)	26.8 (15.9, 58.8)	7.77	0.022	160 (103, 424)	143 (101, 361)	180 (111, 416)	4.46	0.097
rs2282679	AA	CA	CC			AA	CA	CC		
T2D	19.6 (15.2, 39.5)	17.0 (14.4, 28.2)	19.8 (16.8, 37.5)	4.86	0.088	137 (103, 323)	112 (96.1, 183)	123 (100, 252)	7.85	0.020
non-T2D	22.2 (15.4, 52.3)	20.4 (14.6, 44.8)	21.6 (15.8, 54.1)	3.76	0.153	162 (104, 432)	144 (101, 367)	166 (111, 379)	3.77	0.151

SNP: single-nucleotide polymorphism; DBP: Vitamin D binding protein.
Data are expressed as medians (interquartile ranges).
Kruskal-Wallis one-way analysis was performed to evaluate the association between genotypes and vitamin D metabolites concentrations.

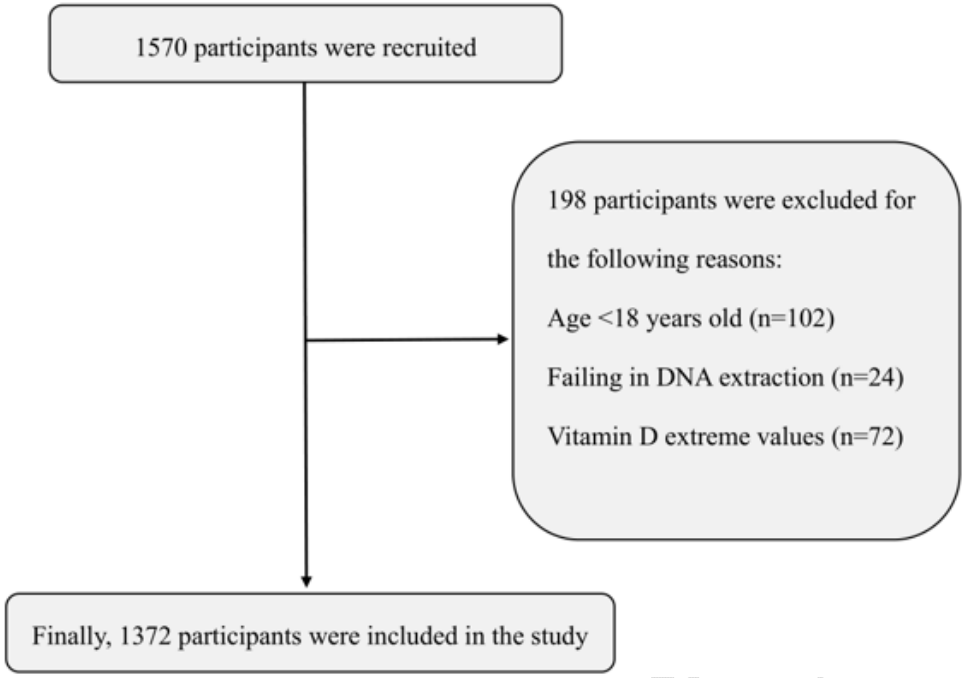


Figure 1. Flow chart of the research object source.

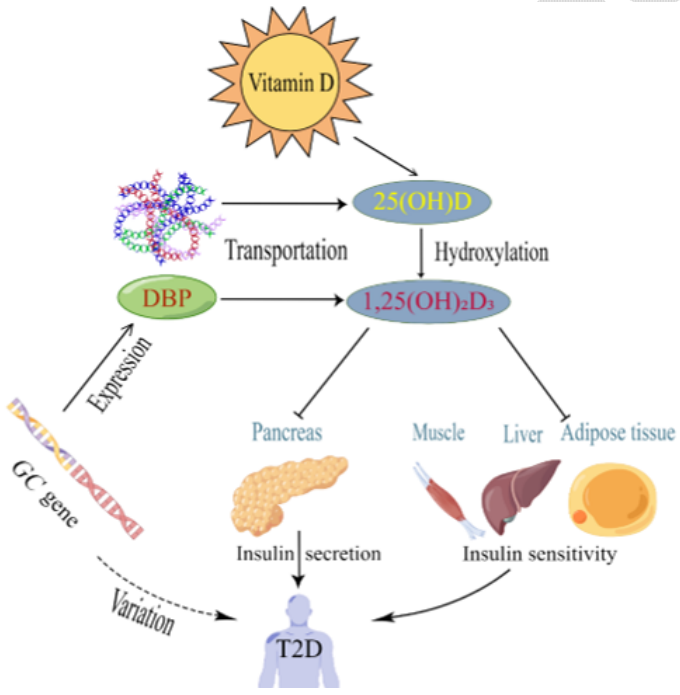
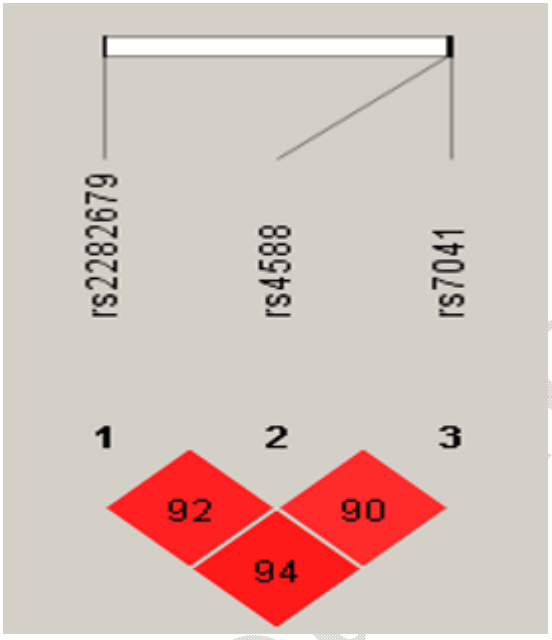


Figure 2. Graphical abstract.

Supplementary table 1. Odds ratios and 95% CI for T2D risk according to vitamin D metabolites concentrations

	Concentration of vitamin D metabolites			
	Q1[†‡]	Q2[†‡]	Q3[†‡]	Q4[†‡]
25(OH)D_3 (ng/ml)				
model 1[§]	1.59 (0.99, 2.56)	1.92 (1.21, 3.06)	1.64 (1.02, 2.63)	Reference
model 2[¶]	1.59 (0.97, 2.60)	2.14 (1.32, 3.46)	1.69 (1.04, 2.77)	Reference
DBP (ng/ml)				
model 1[§]	1.71 (1.07, 2.72)	1.85 (1.16, 2.93)	1.20 (0.74, 1.96)	Reference
model 2[¶]	1.76 (1.09, 2.83)	1.82 (1.13, 2.93)	1.20 (0.73, 1.98)	Reference

DBP: Vitamin D binding protein.
[†]25(OH)D_3 (ng/ml) – Q1 (<15.3); Q2 (15.3–21.0); Q3 (21.0–45.6); Q4 (≥45.6).
[‡]DBP (ng/ml) – Q1 (<104); Q2 (104–149); Q3 (149–368); Q4 (≥368).
[§]Model 1 adjusted for age, gender.
[¶]Model 2 adjusted for age, gender, smoking, drinking, exercise, marry status, occupation, culture and family history of diabetes.



Supplementary figure 1. The results of linkage disequilibrium between the three SNPs.