ORIGINAL RESEARCH

Relationship Between *KCNQ1* Polymorphism and Type 2 Diabetes Risk in Northwestern China

Jing Xu[1]
Wei Zhang[2]
Wei Song [ID][1]
Jiaqi Cui[1]
Yanni Tian[1]
Huan Chen[1]
Pan Huang[1]
Shujun Yang[1]
Lu Wang[1]
Xin He[3]
Lin Wang[4]
Bingyin Shi[5]
Wei Cui [ID][1]

[1]Department of Endocrinology and Second Department of Geriatrics, The First Affiliated Hospital of Xi'an Jiaotong University, Xi'an, Shaanxi, 710061, People's Republic of China; [2]Department of Breast Surgery, The First Affiliated Hospital of Xi'an Jiaotong University, Xi'an, Shaanxi, 710061, People's Republic of China; [3]Department of Endocrinology, Xi'an Aerospace General Hospital, Xi'an, Shaanxi, 710000, People's Republic of China; [4]Department of Endocrinology, Xi'an Gaoxin Hospital, Xi'an, Shaanxi, 710075, People's Republic of China; [5]Department of Endocrinology, The First Affiliated Hospital of Xi'an Jiaotong University, Xi'an, Shaanxi, 710061, People's Republic of China

Correspondence: Wei Cui
The First Affiliated Hospital of Xi'an Jiaotong University, #277 Yanta West Road, Xi'an, 710061, Shaanxi, People's Republic of China
Email doctorweiwei.cui@126.com

Bingyin Shi
The First Affiliated Hospital of Xi'an Jiaotong University, #277 Yanta West Road, Xi'an, 710061, Shaanxi, People's Republic of China
Email xjtougao@126.com

Purpose: This study aimed to explore the relationship between KCNQ1 polymorphism and type 2 diabetes mellitus (T2DM) risk in the population of Northwest China.

Patients and Methods: Case-control strategy was used to reveal the correlation between *KCNQ1* polymorphism and T2DM risk, and MDR analysis clarified the influence of *KCNQ1* polymorphism interaction on T2DM risk. The related proteins, functions, and signal pathways of *KCNQ1* were further explored through bioinformatics methods. PCR was used to explore the relative expression of *KCNQ1* in T2DM patients and the controls.

Results: Studies showed that rs163177, rs163184, rs2237895 and rs2283228 on the *KCNQ1* gene are closely related to the risk of T2DM in Northwest China. MDR results showed that the three-locus model is the best model for T2DM risk assessment, which increases the risk of T2DM. The bioinformatics results showed that *KCNQ1* closely-acted proteins are mainly involved in signal pathways such as gastric acid secretion and renin secretion. The PCR results showed that, compared with the controls, the expression of *KCNQ1* was up-regulated in T2DM patients.

Conclusion: The results revealed that *KCNQ1* polymorphism is related to the risk of T2DM in the population of Northwest China and provide a scientific basis for the early screening and prevention of T2DM high-risk populations.

Keywords: *KCNQ1*, polymorphism, type 2 diabetes, case-control strategy, MDR analysis

Introduction

Type 2 diabetes mellitus (T2DM) is a progressive disease of hyperglycemia characterized by insulin resistance and beta cell dysfunction, which accounts for approximately 85–95% of all diabetes cases.[1,2] In recent years, the number of adults with diabetes in China is among the highest in the world, and its incidence is still rising sharply.[3] The treatment of T2DM imposes enormous social, financial and health system burdens in China. The candidate genes for T2DM have been widely investigated. Numerous single nucleotide polymorphisms (SNPs) have been identified through sequencing, and many of them in critical genes such as *KCNJ11, WFS1, ABCA 1*, and *ALOX5* were demonstrated to be associated with T2DM susceptibility.[4–7] It is well known that different geographic regions can show significant differences in the frequency of certain genetic variations, which lead to differences in susceptibility to disease among populations from different regions. At present, there are many researches on the correlation of T2DM candidate genes in Hong Kong, Shanghai, Jiangsu, Wuhan and other regions of China.[8–12] However, there is very little research

on the susceptibility genes related to T2DM risk in the Northwest region, which accounts for approximately 29% of the total population of China.

Potassium Voltage-Gated Channel Subfamily Q Member 1(*KCNQ1*) is located in a region of chromosome 11p15.5, spanning over 400 kb and containing 676 amino acids. *KCNQ1* encodes a voltage-gated potassium channel required for repolarization phase of the cardiac action potential, and is expressed in the human heart and pancreas and, to a lesser extent, in the placenta, lung liver and kidney.[13] In two independent GWAS studies (2008), *KCNQ1* was first identified as a T2DM susceptibility gene in East Asian populations.[9,10] Subsequently, some studies had confirmed that *KCNQ1* was a susceptibility gene of T2DM in Chinese, Singaporean, Indian and some Euro-Caucasian subjects. In addition, the functional studies on *KCNQ1* have shown that it can stimulate insulin secretion by selective blockade of this K + channel. Furthermore, clinical trait association analysis showed that baseline insulin secretion is impaired in *KCNQ1* risk allele carriers.[10]. All these studies proved that *KCNQ1* plays an important role in the occurrence of T2DM. Studies have shown that *KCNQ1* polymorphism is associated with the risk of T2DM in people in Hong Kong, Shanghai, Jiangsu, Wuhan and other regions of China. However, the correlation between the *KCNQ1* polymorphism and the risk of T2DM in people in Northwestern China has not been reported yet.

Therefore, we conducted a case-control study to explore the influence of *KCNQ1* polymorphism on the risk of T2DM among people in Northwestern China, hoping to provide a genetic theoretical basis for the early screening and prevention of high-risk populations of T2DM in the local area.

Patients and Methods
Study Subjects
Using a case control design, a total of 1011 participants including 508 patients with newly diagnosed T2DM and 503 healthy controls were recruited. All of blood samples of patients were collected from the First Affiliated Hospital of Xi'an Jiaotong University. All the subjects were from a population in Northwest China. We diagnosed patients with T2DM according to the criteria of World Health Organization (WHO)

in 1999: fasting plasma glucose 7.0mmol/L and/or 2 hours postprandial plasma glucose 11.1mmol/L. At the same time, patients with acute diabetes complications, other types of diabetes, type T2DM with lipid-lowering and/or oral hypoglycemic drugs, cardiovascular disease, renal and liver failure, and malignancies were excluded.

The inclusion criteria for unrelated controls were as follows: 1) normal glucose tolerance (fasting plasma glucose < 6.1 mmol/L and 2-h plasma glucose < 7.8 mmol/L), or HbA1c levels < 5.6% with fasting plasma glucose 6.1 mmol/L; 2) no personal or family history of diabetes; and 3) no systemic diseases.

This study was approved by the Ethics Committee of the First Affiliated Hospital of Xi'an Jiaotong University, and in compliance with the Declaration of Helsinki. The purpose of this study was well informed to the all participants and written informed consents were obtained from all participants prior to biological material collection in this study. All subsequent study analyses were conducted in accordance with the approved guidelines and regulations.

Data Collection
Basic information about the subjects was collected by trained professionals using structured questionnaires. The peripheral blood samples from each participant were contained in tubes coating with ethylene-diamine-tetraacetic acid (EDTA) and were stored at − 80°C after centrifugated until analysis. Genomic DNA from whole blood was isolated by the Whole Blood Genomic DNA Extraction Kit (Tiangen Biotech, Beijing, China), and its concentration was measured using the NanoDrop2000 (Thermo Scientific, Waltham, MA, USA).

SNP Selection and Genotyping
Eight tag-SNPs (rs117601636, rs231362, rs231356, rs8181588, rs163177, rs163184, rs2283228, and rs2237895) on the *KCNQ1* gene were selected from the 1000 Genomes Project (http://www.1000genomes.org/) and dbSNP database (https://www. ncbi.nlm.nih.gov/projects/SNP/) for our present study. Minor allele frequencies (MAFs) of these SNPs were > 5% in the Chinese Han population. A multiplexed SNP MassEXTEND assay was designed by Agena MassARRAY Assay Design 3.0 Software (Agena Bioscience, Inc., San Diego, CA, USA). Genotyping of variants was performed by two laboratory personnel in a double-blinded fashion using

the Agena MassARRAY system (Agena, San Diego, CA, U.S.A.),[14,15] and we used Agena Typer 4.0 software for data management and analysis.[16,17] In order to verify the accuracy of genotyping, marker and sample genotyping efficiency as well as the performance of positive and negative controls were examined. In addition, approximately 10% of the total samples were randomly selected for repeated genotyping with a reproducibility of 100%.

Protein-Protein Interaction (PPI), GO and KEGG Analyses

Protein interacting with *KCNQ1* was obtained from the STRING database.[18] Gene ontology (GO) analysis[19] and Kyoto Encyclopedia of Gene and Genome (KEGG) enrichment analysis[20] were performed through the online bioinformatics software (http://www.bioinformatics.com.cn/).

Gene Expression Testing

We collected blood samples from 50 patients and 50 healthy controls who were unrelated to Northwest China for the analysis of *KCNQ1* mRNA expression. According to the manufacturer's instructions, total RNA was extracted from peripheral blood using TRIzol reagent (Invitrogen, Carlsbad, CA, USA). CDNA was obtained by reverse transcription of total RNA using Takara-PrimeScript™ RT Master Mix (Perfect Real Time). Then based on ABI PRISM 7500 Real-Time PCR System (Applied Biosystems), PCR analysis with GAPDH as internal reference was performed. The relative mRNA expression is calculated by the $2^{-\Delta\Delta CT}$ method. The primer sequence is as follows:

KCNQ1-Forward primer: 5′TCTCTGTCTTTGCCAT CTCCTTCTTTG3′

KCNQ1-Reverse primer: 5′CTCCATGCGGTCTGAA TGAGTGAG3′

GAPDH-Forward primer: 5′GGAGCGAGATCCCT CCAAAAT3′

GAPDH–Reverse primer: 5′GGCTGTTGTCATACTT CTCATGG3′

Data Analyses

All the statistical analyses were completed using the Microsoft Excel (Microsoft Corporation, Redmond, WA, USA) and the SPSS 18.0 statistical package (SPSS, Chicago, IL, USA). The differences of basic parameters between the cases and controls were examined with the Pearson's χ^2 tests for categorical variables and independent sample Student t test for continuous variables. For each SNP, Hardy-Weinberg equilibrium (HWE) as well as the differences in allele frequencies and genotype frequencies between cases and controls were examined by χ^2 tests or Fisher's exact test. Beyond that, multiple inheritance model analyses (codominant, dominant, recessive, and log-additive) were generated using PLINK software (http://zzz.bwh.harvard.edu/plink/data.shtml) to estimate the relationship between SNPs and diabetes risk. Furthermore, we also used multiple genetic models to analyze the correlation between SNPs and diabetes risk in different stratified analyses. The diabetes risk linked with alleles and genotypes was estimated by computing the odds ratios (ORs) and their 95% confidence intervals (CIs) from unconditional logistic regression analysis adjusting by gender and age. G*Power 3.1.9.2 was used to calculate the sample size and power value of this study.[21] Exploring the influence of candidate loci on *KCNQ1* gene on T2DM by multi-factor dimensionality reduction (MDR) analysis.[22] The difference in *KCNQ1* mRNA expression between the case group and the control group was evaluated by a test. The relationship between *KCNQ1* polymorphism and *KCNQ1* mRNA expression levels in the case group and the control group was analyzed by one-way analysis of variance. P-value < 0.05 was regarded as statistically significant.

Results
Characteristics of Patients and Controls

G*Power 3.1.9.2 software analysis results showed that the total sample size must be greater than 210 with the sample size of the control group greater than 104, and the sample size of the case group greater than 106 to meet the statistical requirements. The basic characteristics of cases and controls are summarized in Table 1. In this case-control study, a total of 508 patients (277 males and 231 females; age at diagnosis: 59.34 ± 7.62 years) and 503 healthy individuals (279 males and 224 females; age at diagnosis: 59.21 ± 11.90 years) were enrolled, which fully meets the statistical requirements. The efficacy value of this study is 95%, which is in line with statistical significance. There were no statistically significant differences (p = 0.248) in the distributions of gender and age between the case and control groups.

Table 1 Demographic Characteristics of Cases and Controls in This Study

Characteristics	Case	Control	p value
Number	508	503	
Age			> 0.05[a]
≤ 59	245 (48%)	238 (47%)	
> 59	263 (52%)	265 (53%)	
Age (mean ± SD)	59.34 ± 7.62	59.21 ± 11.90	
Gender			> 0.05[b]
Male	277 (55%)	279 (55%)	
Female	231 (45%)	224 (45%)	
Smoking			
Yes	135 (27%)	115 (23%)	
No	230 (45%)	188 (37%)	
Drinking			
Yes	68 (13%)	106 (21%)	
No	277 (55%)	182 (36%)	
BMI index			
≤ 24	130 (26%)	173 (34%)	
> 24	187 (37%)	185 (37%)	

Notes: [a] Two-side Chi-squared test; [b]Independent samples *t*- test; $p < 0.05$ indicates statistical significance.
Abbreviation: BMI, body mass index.

Basic Information of SNPs

Table 2 present the basic information of the eight SNPs of *KCNQ1* in terms of gene, SNP, chromosomal position, role, (MAF of cases and controls, HWE test results and call rate. The call rate for all SNPs was above 99.5% in case and controls, which was considered as high enough to perform association analyses. For all of the *KCNQ1* polymorphisms, the genotype distribution in the control subjects were no deviation from the HWE ($p > 0.05$). We used χ^2 tests to compare the difference in allele frequency between cases and controls, and evaluate the association with the risk of diabetes by ORs. Finally, we found that three significant SNPs in *KCNQ1* were associated with the risk of T2DM. Rs163177 and rs2237895 were associated with a higher T2DM risk in the allele genetic model ($p < 0.05$). Conversely, rs2237895 was associated with a reduced risk of T2DM in the allele genetic model (C vs A: OR = 0.78, 95% CI = 0.65–0.93, $p = 0.007$).

Association Between KCNQ1 and the Risk of Diabetes

Furthermore, we analyzed the association between the eight SNPs and the risk of T2DM under multiple inheritance models (codominant, dominant, recessive, and additive models) (Table 3). As a result, only rs163177,

Table 2 Basic Information and Allele Frequencies About *KCNQ1* Candidate SNPs in This Study

SNP	Chr	Position	Gene(s)	Role	Alleles A/B	Frequency (MAF) Cases	Frequency (MAF) Controls	Call Rate (%)	p-HWE	OR (95% CI)	p value
rs117601636	11	2,620,807	*KCNQ1*	ncRNA exonic	A/G	0.085	0.093	100.00%	0.79	0.90 (0.67–1.23)	0.520
rs231362	11	2,670,241	*KCNQ1*	ncRNA exonic	C/T	0.135	0.126	100.00%	0.685	1.08 (0.84–1.40)	0.546
rs231356	11	2,684,113	*KCNQ1*	ncRNA exonic	A/T	0.216	0.224	99.90%	0.307	0.96 (0.78–1.18)	0.682
rs8181588	11	2,810,311	*KCNQ1*	Intronic	C/T	0.349	0.39	99.80%	0.851	0.84 (0.70–1.01)	0.061
rs163177	11	2,817,183	*KCNQ1*	Intronic	C/T	0.51	0.463	99.70%	0.788	**1.21 (1.01–1.44)**	**0.036** *
rs163184	11	2,825,839	*KCNQ1*	Intronic	G/T	0.493	0.453	99.50%	0.527	1.18 (0.99–1.40)	0.071
rs2283228	11	2,828,300	*KCNQ1*	Intronic	A/C	0.311	0.368	99.60%	0.632	**0.78 (0.65–0.93)**	**0.007** *
rs2237895	11	2,835,964	*KCNQ1*	Intronic	A/C	0.381	0.338	99.60%	0.319	**1.20 (1.00–1.44)**	**0.046** *

Notes: *p*-HWE > 0.05 indicates that the genotypes were in Hardy–Weinberg Equilibrium; *p* values were calculated with Pearson's χ 2 tests; *p* values ≤ 0.05 indicates statistical significance. Data in bold are statistically significant (p < 0.05).
Abbreviations: SNP, single nucleotide polymorphism; Alleles A/B, Minor/Major alleles; MAF, minor allele frequency; OR, odds ratio; CI, confidence interval; HWE, Hardy–Weinberg equilibrium; Chr, chromosome; *KCNQ1*, Potassium Voltage-Gated Channel Subfamily Q Member 1.

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Table 3 Relationship Between *KCNQ1* Gene Polymorphisms and Risk of T2DM Under Multiple Models of Inheritance

SNP	Model	Genotype	Control	Case	Without Adjustment		With Adjustment	
					OR (95% CI)	*p*-value	OR (95% CI)	*p*-value
rs163177	Codominant	T/T	143 (28.4%)	106 (20.9%)	1.00		1.00	
		T/C	254 (50.5%)	285 (56.2%)	1.51 (1.12–2.05)	**0.007 ***	1.52 (1.12–2.05)	**0.007 ***
		C/C	106 (21.1%)	116 (22.9%)	1.48 (1.03–2.12)	**0.036 ***	1.48 (1.03–2.13)	**0.035 ***
	Dominant	T/T	143 (28.4%)	106 (20.9%)	1.00	**0.006 ***	1.00	**0.006 ***
		C/C-T/C	360 (71.6%)	401 (79.1%)	1.5 (1.13–2.01)		1.51 (1.13–2.01)	
	Recessive	T/C-T/T	397 (78.9%)	391 (77.1%)	1.00	0.488	1.00	0.488
		C/C	106 (21.1%)	116 (22.9%)	1.11 (0.82–1.5)		1.11 (0.82–1.50)	
	Log-additive	–	–	–	1.22 (1.02–1.47)	**0.030 ***	1.22 (1.02–1.47)	**0.030 ***
rs163184	Co-dominant	T/T	153 (30.7%)	128 (25.1%)	1.00		1.00	
		G/T	240 (48.1%)	260 (51.1%)	1.3 (0.97–1.74)	0.084	1.3 (0.97–1.74)	0.082
		G/G	106 (21.2%)	121 (23.8%)	1.36 (0.96–1.94)	0.083	1.37 (0.96–1.95)	0.079
	Dominant	T/T	153 (30.7%)	128 (25.1%)	1.00	0.051	1.00	**0.049 ***
		T/G-G/G	346 (69.3%)	381 (74.9%0	1.32 (1.00–1.74)		1.32 (1.00–1.74)	
	Recessive	T/T-T/G	393 (78.8%)	388 (76.2%)	1.00	0.337	1.00	0.329
		G/G	106 (21.2%)	121 (23.8%)	1.16 (0.86–1.56)		1.16 (0.86–1.56)	
	Log-additive	–	–	–	1.18 (0.99–1.4)	0.072	1.18 (0.99–1.40)	0.068
rs2283228	Co-dominant	C/C	197 (39.4%)	239 (47.0%)	1.00		1.00	
		A/C	238 (47.6%)	223 (43.8%)	0.77 (0.59–1.00)	0.054	0.77 (0.59–1.01)	0.055
		A/A	65 (13.0%)	47 (9.2%)	0.60 (0.39–0.91)	**0.016 ***	0.60 (0.39–0.91)	**0.016 ***
	Dominant	C/C	197 (39.4%)	239 (47.0%)	1.00	**0.016 ***	1.00	**0.016 ***
		A/C-A/A	303 (60.6%)	270 (53.0%)	0.73 (0.57–0.94)		0.74 (0.57–0.94)	
	Recessive	C/C-A/C	435 (87.0%)	462 (90.8%)	1.00	0.058	1.00	0.058
		A/A	65 (13.0%)	47 (9.2%)	0.68 (0.46–1.01)		0.68 (0.46–1.01)	
	Log-additive	–	–	–	0.77 (0.64–0.93)	**0.007 ***	0.77 (0.64–0.93)	**0.007 ***
rs2237895	Co-dominant	C/C	214 (42.7%)	193 (38.0%)	1.00		1.00	
		A/C	235 (46.9%	243 (47.8%)	1.15 (0.88–1.49)	0.311	1.15 (0.88–1.50)	0.309
		A/A	52 (10.2%)	72 (34.6%)	1.54 (1.02–2.31)	**0.039 ***	1.53 (1.02–2.30)	**0.039 ***

(*Continued*)

Table 3 (Continued).

SNP	Model	Genotype	Control	Case	Without Adjustment		With Adjustment	
					OR (95% CI)	p-value	OR (95% CI)	p-value
	Dominant	C/C	214 (42.7%)	193 (38.0%)	1.00		1.00	0.126
		A/C-A/A	287 (57.3%)	315 (62.0%)	1.22 (0.95–1.57)	0.127	1.22 (0.95–1.57)	
	Recessive	C/C-A/C	449 (89.8%)	436 (65.4%)	1.00		1.00	0.068
		A/A	52 (10.2%)	72 (34.6%)	1.43 (0.97–2.09)	0.067	1.42 (0.97–2.08)	
	Log-additive	–	–	–	1.21 (1.01–1.46)	**0.044 ***	1.21 (1.01–1.46)	**0.044 ***

Notes: p values were calculated with Pearson's χ 2 tests; *p value < 0.05 indicates statistical significance (p < 0.05) are in bold.
Abbreviations: ORs, odds ratios; 95% CI, 95% confidence interval; *KCNQ1*, Potassium Voltage-Gated Channel Subfamily Q Member 1; T2DM, Type 2 diabetes mellitus.

rs2237895, rs163184, and rs2283228 were found to be associated with the risk of T2DM. The results showed that rs163177 significantly increased the risk of T2DM under the codominant, dominant and log-additive models (p < 0.05). The SNP locus rs2237895 was significantly correlated with an increased risk of T2DM under the codominant and log-additive models (A/A vs CC: OR = 1.53, 95% CI = 1.02–2.30, p = 0.039; A vs C: OR = 1.21, 95% CI = 1.01–1.46, p = 0.044). The SNP locus rs163184 only increased the risk of T2DM in the dominant model (T/G-G/G vs TT: OR = 1.30, 95% CI = 1.00–1.74, p = 0.049). However, rs2283228 polymorphism had a significantly reduced risk of T2DM based on the codominant, dominant, and log-additive models (p < 0.05).

Stratification Analysis by Age and Gender

We performed a subgroup analysis to evaluate the effect of the SNPs on T2DM stratified by age adjusted for age and gender. As shown in Table 4, the results indicated that rs163177 was associated with an increased T2DM risk in patients at age ≤ 59 years in codominant model, and dominant models (TC vs TT, OR = 1.74, 95% CI = 1.13–2.69, p = 0.012; C/C - T/C vs TT, OR = 1.65, 95% CI = 1.08–2.54, p = 0.022). Rs8181588 polymorphism was observed to be associated with the reduced susceptibility of T2DM in patients at age > 59 years under the allele model (C vs T, OR = 0.78, 95% CI = 0.60–1.00, p = 0.046). Meanwhile, rs2283228 also showed a negative effect on T2DM at age in patients > 59 years under the log-additive (A vs C, OR = 0.76, 95% CI: 0.58–0.99, p = 0.041) and allele (A vs C, OR= 0.74, 95% CI: 0.58–0.96, p = 0.024) model.

Stratified analysis by gender adjusted for age is also revealed significant associations between three SNPs and the risk of T2DM as presented in Table 4. Rs163177 polymorphism was significantly associated with increasing the T2DM risk among the female subgroup under the codominant, dominant, log-additive, and allele models (p < 0.05). Rs2237895 polymorphism also exhibited an increased T2DM risk among female in the co-dominant, recessive, log-additive, and allele models (p < 0.05). Conversely, rs2283228 polymorphism was associated with a reduced susceptibility of diabetes in female under the codominant and dominant models (p < 0.05). However, there was no relationship existed between the selected SNPs and diabetes risk in male.

Table 4 Relationship of *KCNQ1* Gene Polymorphisms and Risk of T2DM Stratified by Gender and Age (Adjusted by Sex, Age)

SNP	Model	Genotype	Age (Years)				Gender			
			Age (Years) ≤ 59		Age (Years) > 59		Male		Female	
			OR (95% CI)	*p* value	OR (95% CI)	*p* value	OR (95% CI)	*p* value	OR (95% CI)	*p* value
rs8181588	Co-dominant	T/T	1.00		1.00		1.00		1.00	
		T/C	0.83 (0.57–1.22)	0.344	0.9 (0.61–1.32)	0.573	0.95 (0.66–1.35)	0.759	0.74 (0.50–1.10)	0.133
		C/C	0.91 (0.51–1.64)	0.762	0.6 (0.34–1.04)	0.067	0.68 (0.41–1.15)	0.150	0.72 (0.40–1.32)	0.292
	Dominant	T/T	1.00	0.490	1.00	0.268	1.00	0.446	1.00	0.111
		C/C-T/C	0.87 (0.60–1.28)		0.81 (0.57–1.17)		0.88 (0.62–1.23)		0.74 (0.50–1.07)	
	Recessive	T/C-T/T	1.00	0.865	1.00	0.081	1.00	0.154	1.00	0.59
		C/C	0.95 (0.54–1.69)		0.63 (0.38–1.06)		0.70 (0.43–1.14)		0.86 (0.49–1.50)	
	Log-additive	—	0.92 (0.69–1.22)	0.554	0.8 (0.62–1.04)	0.093	0.86 (0.67–1.09)	0.206	0.82 (0.62–1.08)	0.153
	Allele	T	1.00	0.532	1.00	0.046 *	1.00	0.203	1.00	0.160
		C	0.92 (0.71–1.20)		0.78 (0.60–1.00)		0.85 (0.67–1.09)		0.82 (0.63–1.08)	
rs163177	Codominant	T/T	1.00		1.00		1.00		1.00	
		T/C	1.74 (1.13–2.69)	0.012 *	1.26 (0.81–1.96)	0.297	1.56 (1.03–2.35)	0.035	1.46 (0.93–2.28)	0.101
		C/C	1.63 (0.96–2.74)	0.068	1.23 (0.72–2.08)	0.446	1.25 (0.76–2.04)	0.381	1.81 (1.05–3.13)	0.033 *
	Dominant	T/T	1.00	0.022 *	1.00	0.292		0.06	1.00	0.045 *
		C/C-T/C	1.65 (1.08–2.54)		1.25 (0.82–1.90)		1.46 (0.98–2.16)		1.55 (1.01–2.38)	
	Recessive	T/C-T/T	1.00	0.740	1.00	0.846	1.00	0.679	1.00	0.140
		C/C	1.08 (0.69–1.69)		1.04 (0.68–1.60)		0.92 (0.62–1.37)		1.41 (0.89–2.21)	
	Log-additive	–	1.26 (0.96–1.65)	0.098	1.11 (0.85–1.45)	0.434	1.12 (0.88–1.44)	0.350	1.35 (1.03–1.77)	0.030 *
	Allele	T	1.00	0.059	1.00	0.296	1.00	0.370	1.00	0.038 *
		C	1.28 (0.99–1.64)		1.14 (0.89–1.45)		1.11 (0.88–1.41)		1.32 (1.02–1.71)	
rs2283228	Co-dominant	C/C	1.00		1.00		1.00		1.00	
		A/C	0.75 (0.52–1.10)	0.141	0.77 (0.53–1.12)	0.174	0.87 (0.61–1.23)	0.423	0.67 (0.45–0.99)	0.045 *
		A/A	0.7 (0.37–1.31)	0.259	0.57 (0.32–1.02)	0.059	0.56 (0.31–0.98)	0.044 *	0.66 (0.35–1.23)	0.187
	Dominant	C/C	1.00	0.149	1.00	0.073	1.00	0.185	1.00	0.033 *
		A/C-A/A	0.76 (0.52–1.11)		0.72 (0.5–1.03)		0.8 (0.57–1.12)		0.67 (0.46–0.97)	
	Recessive	C/C-A/C	1.00	0.381	1.00	0.127	1.00	0.064	1.00	0.468
		A/A	0.76 (0.40–1.42)		0.65 (0.37–1.13)		0.6 (0.35–1.03)		0.8 (0.45–1.45)	

(*Continued*)

Table 4 (Continued).

SNP	Model	Genotype	Age (Years)				Gender			
			Age (Years) ≤ 59		Age (Years) > 59		Male		Female	
			OR (95% CI)	p value	OR (95% CI)	p value	OR (95% CI)	p value	OR (95% CI)	p value
	Log-additive	—	0.8 (0.60–1.07)	0.131	0.76 (0.58–0.99)	**0.041 ***	0.78 (0.61–1.01)	0.060	0.76 (0.58–1.01)	0.054
	Allele	C A	1.00 0.81 (0.62–1.07)	0.133	1.00 0.74 (0.58–0.96)	**0.024 ***	1.00 0.79 (0.62–1.01)	0.064	1.00 0.76 (0.58–1.01)	0.054
rs2237895	Co-dominant	C/C A/C A/A	1.00 1.19 (0.81–1.74) 1.46 (0.81–2.63)	0.378 0.209	1.07 (0.73–1.57) 1.61 (0.9–2.86)	0.731 0.109	1.24 (0.72–2.15) 1.07 (0.75–1.53)	0.441 0.695	1.25 (0.84–1.86) 2.04 (1.11–3.76)	0.270 **0.023 ***
	Dominant	C/C A/C-A/A	1.00 1.14 (0.78–1.66)	0.501	1.00 1.16 (0.81–1.67)	0.413	1.00 1.11 (0.79–1.55)	0.564	1.00 1.38 (0.95–2.02)	0.091
	Recessive	C/C-A/C A/A	1.00 1.23 (0.69–2.19)	0.486	1.00 1.55 (0.90–2.65)	0.113	1.00 1.19 (0.71–1.99)	0.500	1.00 1.81 (1.02–3.21)	**0.043 ***
	Log-additive	—	1.13 (0.85–1.49)	0.404	1.21 (0.93–1.57)	0.166	1.10 (0.86–1.42)	0.450	1.37 (1.04–1.81)	**0.026 ***
	Allele	C A	1.00 1.2 (0.92–1.56)	0.183	1.00 1.22 (0.95–1.56)	0.129	1.00 1.1 (0.86–1.40)	0.464	1.00 1.36 (1.03–1.78)	**0.028 ***

Notes: *p value < 0.05 indicates statistical significance (p < 0.05) are in bold. p values were calculated by unconditional logistic regression analysis with adjustments for age.
Abbreviations: ORs, odds ratios; 95% CI, 95% confidence interval; KCNQ1, Potassium Voltage-Gated Channel Subfamily Q Member 1; T2DM, type 2 diabetes mellitus.

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Table 5 Relationship of *KCNQ1* Gene Polymorphisms and Risk of T2DM Stratified by Smoking and Drinking (Adjusted by Sex, Age)

SNP	Model	Genotype	Smokers		Nondrinkers		Drinkers		Without Drinking History	
			OR (95% CI)	p value	OR (95% CI)	p value	OR (95% CI)	p value	OR (95% CI)	p value
rs231362	Co-dominant	T/T T/C C/C	1.00 0.73 (0.33–1.57) 0.89 (0.11–7.33)	0.416 0.912	1.00 1.76 (1.05–2.98) 3.20 (0.35–29.56)	**0.034 *** 0.305	1.00 0.52 (0.04–6.03) 0.31 (0.12–0.83)	0.599 **0.020 ***	1.00 2.01 (1.20–3.35) 3.63 (0.40–32.74)	**0.008 *** 0.251
	Dominant	T/T C/C-T/C	1.00 0.74 (0.35–1.56)	0.427	1.00 1.81 (1.08–3.04)	**0.023 ***	1.00 0.33 (0.13–0.83)	**0.019 ***	1.00 2.06 (1.25–3.42)	**0.005 ***
	Recessive	T/C-T/T C/C	1.00 0.96 (0.12–7.87)	0.969	1.00 2.79 (0.30–25.93)	0.367	1.00 0.65 (0.06–7.57)	0.734	1 3.11 (0.34–28.20)	0.314
	Log-additive	—	0.79 (0.41–1.52)	0.481	1.77 (1.09–2.87)	**0.021 ***	0.40 (0.18–0.93)	**0.033 ***	1.99 (1.24–3.20)	**0.005 ***
	Allele	T C	1.00 1.10 (0.65–1.85)	0.719	1.00 1.42 (0.96–2.11)	0.081	1.00 0.55 (0.28–1.09)	0.084	1.00 1.53 (1.04–2.25)	**0.032 ***
rs231356	Co-dominant	T/T T/A A/A	1.00 0.91 (0.45–1.86) 0.92 (0.21–3.99)	0.795 0.913	1.00 1.05 (0.67–1.63) 2.35 (0.72–7.63)	0.849 0.157	1.00 0.39 (0.17–0.89) 0.40 (0.07–2.27)	**0.024 *** 0.301	1.00 1.27 (0.82–1.97) 2.79 (0.87–8.93)	0.279 0.084
	Dominant	T/T A/A-T/A	1.00 0.91 (0.46–1.8)	0.789	1.00 1.13 (0.73–1.75)	0.572	1.00 0.39 (0.18–0.85)	**0.018 ***	1.00 1.38 (0.90–2.11)	0.136
	Recessive	T/A-T/T A/A	1.00 0.95 (0.23–4.04)	0.950	1.00 2.31 (0.72–7.43)	0.161	1.00 0.56 (0.10–3.08)	0.503	1.00 2.56 (0.81–8.12)	0.110
	Log-additive	—	0.93 (0.54–1.63)	0.809	1.21 (0.83–1.74)	0.324	0.48 (0.25–0.93)	0.030 *	1.41 (0.98–2.02)	0.066
	Allele	T A	1.00 1.12 (0.74–1.72)	0.588	1.00 1.05 (0.76–1.46)	0.753	1.00 0.61 (0.36–1.04)	0.068	1.00 1.19 (0.86–1.63)	0.297
rs2283228	Co-dominant	C/C A/C A/A	1 0.60 (0.3–1.23) 1.03 (0.31–3.4)	0.162 0.963	1.00 0.83 (0.54–1.30) 0.58 (0.27–1.22)	0.418 0.150	1.00 0.65 (0.31–1.36) 0.44 (0.08–2.51)	0.25 0.359	1.00 0.79 (0.51–1.22) 0.72 (0.36–1.42)	0.289 0.340
	Dominant	C/C A/C-A/A	1.00 0.66 (0.33–1.31)	0.233	1.00 0.78 (0.51–1.18)	0.244	1.00 0.62 (0.30–1.29)	0.201	1.00 0.78 (0.51–1.17)	0.224
	Recessive	C/C-A/C A/A	1.00 1.35 (0.44–4.16)	0.602	1.00 0.63 (0.31–1.29)	0.204	1.00 0.55 (0.10–3.01)	0.494	1.00 0.80 (0.42–1.54)	0.505

(*Continued*)

Table 5 (Continued).

SNP	Model	Genotype	Smokers		Nondrinkers		Drinkers		Without Drinking History	
			OR (95% CI)	p value	OR (95% CI)	p value	OR (95% CI)	p value	OR (95% CI)	p value
	Log-additive	—	0.84 (0.50–1.40)	0.506	0.79 (0.57–1.09)	0.145	0.65 (0.35–1.22)	0.182	0.83 (0.61–1.12)	0.223
	Allele	C	1.00	0.868	1.00		1.00	0.260	1.00	
		A	0.97 (0.67–1.40)		0.74 (0.55–0.99)	**0.043 ***	0.77 (0.48–1.22)		0.82 (0.61–1.08)	0.157

Notes: p values were calculated by unconditional logistic regression analysis with adjustments for age; *p value < 0.05 indicates statistical significance (p < 0.05) are in bold.
Abbreviations: ORs, odds ratios; 95% CI, 95% confidence interval; KCNQ1, Potassium Voltage-Gated Channel Subfamily Q Member 1; T2DM, type 2 diabetes mellitus.

Stratification Analysis by Smoking and Alcohol Drinking

We further explored the potential interactions between the selected SNPs and the development of T2DM based on smoking and drinking stratification, and found the results in Table 5. According to the stratification of smoking status, it was found that rs231362 can increase the susceptibility of non-smokers to T2DM under the co-dominant, dominant, and log-additive models ($p < 0.05$). For rs2283228, the susceptibility of nonsmokers to T2DM in the allele model was reduced (A vs C, OR=0.74, 95% CI: 0.55–0.99, $p = 0.043$).

When stratified by drinking status, rs231362 was observed to decrease the susceptibility to T2DM among individuals without drinking history based on the codominant, dominant, log-additive and allele models ($p < 0.05$). Both rs231362 and rs231356 were associated with reduced risks of T2DM under the codominant, dominant, and recessive models among drinkers ($p < 0.05$).

Stratification Analysis by Body Mass Index (BMI)

Finally, when stratified analysis according to BMI, we found that four SNPs loci were significantly correlated with T2DM risk, as listed in Table 6. Rs231362 polymorphism was associated with a higher T2DM risk in allele genetic models at BMI < 24 (A vs C: OR = 1.67, 95% CI = 1.02–2.72, $p = 0.039$). Rs163177 and rs163184 polymorphisms also increased the risk of T2DM in the codominant, dominant, log-additive, allele models at BMI < 24 ($p < 0.05$). Rs2283228 polymorphism was associated with a decreased risk of T2DM under the log-additive and allele models at BMI < 24 (log-additive model: A vs C: OR = 0.67, 95% CI = 0.45–0.98, $p = 0.041$; allele model: A vs C: OR = 0.67, 95% CI = 0.48–0.95, $p = 0.025$).

Relationship Between the Genotype of KCNQ1 SNPs and Clinical Indicators in Patients with T2DM

Studies have shown that the study of clinical indicators of T2DM is of great significance for the clinical treatment of T2DM.[23,24] We also analyzed the relationship between eight SNPs in KCNQ1 gene and the clinical parameters of T2DM, including fasting glucose, glycosylated hemoglobin, total cholesterol, triglyceride, LDL, HDL and urea, and the positive results are listed in Table 7. We found that

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Table 6 Relationship of *KCNQ1* Gene Polymorphisms and Risk of T2DM Stratified by BIM (Adjusted by Sex, Age)

SNP	Model	Genotype	BMI < 24				BMI ≥ 24			
			Control	Case	OR (95% CI)	p-value	Control	Case	OR (95% CI)	p-value
rs231362	Co-dominant	T/T	142 (82.1%)	92 (70.8%)	1.00		137 (74.1%)	136 (72.7%)	1.00	
		T/C	28 (16.2%)	36 (27.7%)	1.82 (0.97–3.43)	0.063	44 (23.8%)	47 (25.2%)	0.91 (0.51–1.61)	0.747
		C/C	3 (1.7%)	2 (1.5%)	1.36 (0.19–10)	0.760	4 (2.2%)	4 (2.1%)	2.84 (0.28–28.71)	0.377
	Dominant	T/T	142 (82.1%)	92 (70.8%)	1.00	0.064	137 (74.1%)	136 (72.7%)	1.00	0.904
		C/C-T/C	31 (17.9%)	38 (29.2%)	1.79 (0.97–3.29)		48 (25.9%)	51 (27.3%)	0.97 (0.55–1.69)	
	Recessive	T/C-T/T	170 (98.3%)	128 (98.5%)	1.00	0.857	181 (97.8%)	183 (97.9%0	1.00	0.891
		C/C	3 (1.7%)	2 (1.5%)	1.2 (0.16–8.76)		4 (2.2%)	4 (2.1%)	1.04 (0.62–1.73)	
	Log-additive	–	–	–	1.62 (0.93–2.82)	0.087	–	–	2.91 (0.29–29.28)	0.364
	Allele	T	148 (86.0%)	148 (86.0%)	1.00	**0.039** *	318 (85.6%)	319 (85.3%0	1.00	0.800
		C	24 (14.0%)	24 (14.0%)	1.67 (1.02–2.72)		52 (14.1%)	55 (14.7%)	1.05 (0.7–1.59)	
rs163177	Codominant	T/T	47 (27.2%0	20 (15.4%)	1.00		51 (27.6%)	47 (25.3%)	1.00	
		T/C	86 (49.7%)	72 (655.4%)	1.81 (0.92–3.57)	0.087	94 (20.8%)	99 (53.8%)	0.75 (0.40–1.39)	0.358
		C/C	40 (23.1%)	38 (29.2%)	2.27 (1.05–4.90)	**0.037 ***	40 (21.6%)	40 (21.5%)	0.85 (0.40–1.82)	0.682
	Dominant	T/T	47 (27.2%0	20 (15.4%)	1.00	**0.043 ***	51 (27.6%)	47 (25.3%)	1.00	0.403
		C/C-T/C	126 (72.8%)	110 (84.6%0	1.95 (1.02–3.73)		145	146	0.78 (0.43–1.41)	
	Recessive	T/C-T/T	133 (76.9%)	92 (70.8%)	1.00	0.194	145 (48.4%)	146 (79.1%)	1.00	0.877
		C/C	40 (23.1%)	38 (29.2%)	1.48 (0.82–2.65)		40 (21.6%)	40 (21.5%)	1.05 (0.57–1.94)	
	Log-additive	–	–	–	1.49 (1.01–2.18)	**0.042 ***	–	–	0.92 (0.63–1.34)	0.662
	Allele	T	180 (52.0%)	112 (43.1)	1	**0.029 ***	196 (53.0%)	193 (51.9%)	1.00	0.766
		C	166 (48.0%0	148 (56.9%)	1.43 (1.04–1.98)		174 (47.0%)	179 (48.1%)	1.05 (0.78–1.39)	
rs163184	Co-dominant	T/T	54 (31.8%)	26 (20.0%)	1.00		51 (27.7%)	51 (27.3%)	1.00	
		G/T	77 (45.3%)	68 (52.3%)	1.92 (1.02–3.65)	**0.045 ***	92 (50.0%)	96 (51.3%)	0.76 (0.41–1.41)	0.378
		G/G	39 (22.9%)	36 (27.7%)	2.07 (1.00–4.27)	**0.049 ***	41 (22.3%)	40 (21.4%)	0.81 (0.39–1.71)	0.584
	Dominant	T/T	54 (31.8%)	26 (20.0%)	1.00	**0.026 ***	51 (27.7%)	51 (27.3%)	1.00	0.392
		T/G-G/G	116 (68.2%)	104 (80.0%)	1.97 (1.09–3.59)		133 (72.3%)	136 (72.7%)	0.77 (0.43–1.40)	
	Recessive	T/T-T/G	131 (12.9%)	94 (72.3%)	1.00	0.313	143 (77.7%0	147 (78.6%)	1.00	0.961
		G/G	39 (22.9%)	36 (27.7%)	1.36 (0.75–2.45)		41 (22.3%)	40 (21.4%)	0.98 (0.54–1.81)	
	Log-additive	–	–	–	1.44 (1.00–2.07)	**0.049 ***	–	–	0.9 (0.62–1.30)	0.567

(Continued)

Table 6 (Continued).

SNP	Model	Genotype	BMI < 24				BMI ≥ 24			
			Control	Case	OR (95% CI)	*p*-value	Control	Case	OR (95% CI)	*p*-value
	Allele	T G	185 (54.4%) 155 (45.6%0	120 (46.2%) 140 (53.8%)	 1.39 (1.01–1.93)	**0.045 ***	194 (52.7%) 174 (47.3%)	198 (52.9%) 176 (47.1%)	 0.99 (0.74–1.32)	0.951
rs2283228	Co-dominant	C/C A/C A/A	66 (38.4%) 82 (47.6%) 24 (14.0%)	66 (51.2) 51 (39.5%) 12 (9.3%)	1.00 0.69 (0.40–1.21) 0.43 (0.18–1.02)	 0.194 0.056	81 (44.0%) 84 (45.7%) 19 (10.3%)	92 (49.2%) 77 (41.2%0 18 (9.6%)	1.00 0.83 (0.49–1.38) 1.31 (0.47–3.68)	 0.467 0.609
	Dominant	C/C A/C-A/A	66 (38.4%) 106 (61.6%)	66 (51.2) 63 (48.8%)	1.00 0.63 (0.37–1.06)	0.080	81 (44.0%) 103 (56%)	92 (49.2%) 95 (50.8%)	1.00 0.88 (0.54–1.45)	0.614
	Recessive	C/C-A/C A/A	148 (86.0%) 24 (14.0%)	117 (90.7%) 12 (9.3%)	1.00 0.51 (0.22–1.17)	0.112	165 (89.7%) 19 (10.3%)	169 (90.4%) 18 (9.6%)	1.00 1.43 (0.53–3.91)	0.483
	Log-additive	–	–	–	0.67 (0.45–0.98)	**0.041 ***	–	–	0.98 (0.66–1.45)	0.913
	Allele	C A	214 (62.2%) 130 (37.8%)	183 (70.9%) 75 (29.1%0	1.00 0.67 (0.48–0.95)	**0.025 ***	246 (66.8%) 122 (33.2%)	261 (69.8%) 113 (30.2%)	1.00 0.87 (0.64–1.19)	0.390

Notes: *p* values were calculated by unconditional logistic regression analysis with adjustments for age; *p value < 0.05 indicates statistical significance (*p* < 0.05) are in bold.
Abbreviations: ORs, odds ratios; 95% CI, 95% confidence interval; BMI, body mass index; *KCNQ1*, Potassium Voltage-Gated Channel Subfamily Q Member 1; T2DM, type 2 diabetes mellitus.

"GA", "AG" and "AA" genotypes of rs117601636 were significantly associated with total cholesterol and LDL levels. The genotype "AA" of rs231362 carriers has the higher ubiquitin cross-reacting protein (UCRP) and TJCTNT levels than "AG" and "AA" genotype carriers. For locus rs8181588, "CC" carriers were observed to have higher total cholesterol, LDL, and ALBP levels than "CA" and "AA" carriers. The "TT", "GT", and "GG" genotypes of rs163177 were significantly correlated with total cholesterol and INS (insulin) content. The "CC", "CA", and "AA" genotypes of rs2237895 were significantly related to LDL content. There also was a significant correlation between the three genotypes of rs2237895 and LDL content. Compared to those with "CC" and "AA" genotypes of rs2237895, the LDL levels of "CA" genotype carriers were higher.

MDR Analysis for the Effect of KCNQ1 SNP-SNP Interaction on T2DM Risk

The MDR analysis method explored the influence of *KCNQ1* SNP-SNP interaction on the risk of T2DM. The dendrogram (Figure 1A) and fruchterman Rheingold (Figure 1B) show that the interaction of rs117601636 and rs231362 on the *KCNQ1* gene has a strong synergistic effect, while the interaction of rs163177 and rs2283228 on the *KCNQ1* gene a strong antagonistic effect. The best locus model of *KCNQ1* SNPs' susceptibility to T2DM is shown in Table 8. Among all models, the combination of rs2237895, rs2283228, rs231356, rs117601636, rs163177 and rs8181588 is the best predictive model for T2DM risk (testing accuracy = 0.568, cross-validation consistency (CVC) = 10/10, OR = 4.10, 95% CI = 3.12–5.39, $p < 0.001$).

PPI, GO, and KEGG Analyses

The above studies indicate that *KCNQ1* polymorphism is associated with the risk of T2DM. In order to further clarify how *KCNQ1* acts on T2DM, this study conducted PPI, GO, and KEGG analyses. PPI results showed that the proteins strongly related to *KCNQ1* are KCNE2, KCNE1L, CALM3, CALM2, CALM1, AKAP9, KCNJ2, KCNE4, especially KCNE1 and KCNE3 (Figure 2). From those GO terms, the main proteins related to *KCNQ1* are mainly involved in the regulation of heart rate by cardiac conduction, voltage-gated potassium channel complex, ion channel binding, and N-terminal myristoylation domain binding (Figure 3). From those KEGG terms, the main

pathways involved in the main proteins related to *KCNQ1* are gastric acid secretion, renin secretion, adrenergic signaling in cardiomyocytes and oxytocin signaling pathway (Figure 4).

Relative Expression of KCNQ1 mRNA in the Cases and the Controls

Based on the above research situation, we used real-time quantitative PCR method to detect the relative expression of *KCNQ1* at the mRNA level in the case group and the control group. Although there was no significant statistical difference in the mRNA expression level of *KCNQ1* between the two groups, we did observe that in a single sample, the expression of *KCNQ1* was up-regulated in the cases relative to the controls ($p > 0.05$) (Figure 5).

Relationship Between KCNQ1 mRNA Expression and Its Polymorphism

At the mRNA level, there was no statistical correlation between the expression of *KCNQ1* in patients and controls and the genotypes of candidate SNPs on *KCNQ1* (rs117601636, rs231362, rs231356, rs8181588, rs163177, rs163184, rs2283228, and rs2237895) ($p > 0.05$) (Figure 6).

Discussion

This study explored the correlation between *KCNQ1* polymorphism and the risk of T2DM in Northwest China. The overall analysis results show that rs163177, rs163184 and rs2237895 are associated with increased risks of T2DM in people from Northwestern China, but rs2283228 is associated with a lower risk. In addition, stratified analysis showed that age, gender, smoking status, drinking status, and BMI were all affected the correlation between *KCNQ1* polymorphism and the risk of T2DM in the population of Northwest China. MDR analysis showed that the best single-locus model is rs2283228, and the best multi-locus model is the six-locus model. In addition, we found that the expression of *KCNQ1* is down-regulated in T2DM patients relative to healthy controls. To our knowledge, this is the first study to evaluate the relationship between *KCNQ1* polymorphism and T2DM risk in Northwest China.

KCNQ1 is closely related to the occurrence of T2DM. The evidence to date demonstrated that common variants of *KCNQ1* could mediate the susceptibility of people of

Table 7 The Relationship Between *KCNQ1* Genotype and Clinical Indicators of T2DM

SNP	Genotype	Total Cholesterol	Triglycerides	LDL	HDL	ALBP	INS	UCRP	TJCTNT
rs117601636	AA	4.57±1.34	2.6±2.37	2.65±1.05	1.67±8.42	41.24±132.36	19.29±19.75	0.58±1.37	0.01±0.01
	GA	4.27±0.97	2.01±1.63	2.42±0.82	1.18±0.35	14.41±19.65	16.53±11.09	0.36±0.66	0.01±0
	GG	5.79±3.05	2.28±1.84	3.61±2.33	1.34±0.51	5.32±2.27	13.78±13.38	0.48±0.63	0.01±0
	p	**0.036 ***	0.224	**0.043 ***	0.895	0.432	0.585	0.527	0.271
rs231362	GG	4.56±1.35	2.6±2.38	2.62±1.04	1.19±0.58	43.26±140.4	19.28±19.35	0.58±1.39	0.01±0.01
	AG	4.46±1.24	2.25±1.99	2.65±1.08	2.64±14.68	17.99±28.82	18.03±17.19	0.35±0.6	0.01±0
	AA	4.54±1.26	2.14±1.31	2.52±0.94	1.2±0.32	35.99±59.34	10.83±3.62	1.84±2.79	0.02±0.03
	p	0.813	0.438	0.53	0.249	0.37	0.558	**0.015 ***	**0.001 ***
rs231356	TT	4.57±1.37	2.74±2.55	2.61±1.01	1.2±0.65	47.4±151.48	19.59±20.18	0.52±1.13	0.01±0.01
	AT	4.48±1.28	2.2±1.81	2.65±1.14	2.3±12.98	19.02±29.39	18.93±16.7	0.54±1.41	0.01±0.01
	AA	4.38±0.93	1.75±0.87	2.5±0.7	1.25±0.3	20.87±37.59	9.49±5.92	0.82±1.82	0.01±0.02
	p	0.685	0.052	0.802	0.406	0.206	0.116	0.664	0.472
rs8181588	TT	4.45±1.26	2.32±2.16	2.55±0.9	1.24±0.59	23.53±53.5	18.02±20.72	0.51±1.41	0.01±0.01
	CT	4.55±1.3	2.41±1.89	2.67±1.14	1.16±0.44	39.25±96.25	18.49±15.72	0.6±1.23	0.01±0
	CC	4.83±1.61	3.76±3.64	2.68±1.16	4.81±23.44	89.42±305.03	23.76±20.58	0.44±0.67	0.01±0.01
	p	0.226	**0.006 ***	0.547	**0.015 ***	**0.040 ***	0.317	0.746	0.871
rs163177	TT	4.53±1.32	2.91±2.59	2.54±0.81	1.27±0.77	39.49±70.33	23.77±24.17	0.75±1.36	0.01±0
	CT	4.54±1.36	2.6±2.35	2.65±1.18	1.84±10.21	45.72±159.06	16.65±12.19	0.5±1.34	0.01±0.01
	CC	4.55±1.21	1.96±1.65	2.61±0.85	1.25±0.36	14.82±16.42	19.32±23.74	0.47±1.06	0.01±0.01
	p	0.993	**0.038 ***	0.692	0.756	0.261	**0.039 ***	0.384	0.411
rs163184	TT	4.51±1.38	2.88±2.7	2.47±0.85	2.82±15.42	41.16±68.58	21.56±22.5	0.77±1.51	0.01±0
	GT	4.52±1.34	2.57±2.28	2.68±1.19	1.15±0.58	45.57±161.67	16.81±12.79	0.47±1.25	0.01±0.01
	GG	4.59±1.22	1.99±1.65	2.63±0.85	1.26±0.36	13.63±14.09	20.25±23.98	0.47±1.06	0.01±0.01
	p	0.897	0.051	0.264	0.188	0.233	0.165	0.243	0.185
rs2283228	CC	4.67±1.29	3.17±2.9	2.61±0.69	5.79±26.86	27.66±35.16	25.41±20.86	0.54±0.73	0.01±0.01
	CA	4.53±1.4	2.59±2.39	2.62±1.2	1.19±0.63	54.34±179.24	17.51±15.08	0.59±1.28	0.01±0
	AA	4.51±1.24	2.32±2.04	2.6±0.92	1.23±0.56	24.71±54.92	18.82±20.72	0.51±1.35	0.01±0.01
	p	0.811	0.198	0.987	**0.005 ***	0.169	0.169	0.89	0.784
rs2237895	CC	4.51±1.36	2.23±2.09	2.53±0.84	1.23±0.41	16.24±17.57	21.84±28.94	0.51±0.96	0.01±0.01
	CA	4.63±1.32	2.58±2.41	2.75±1.21	1.99±11.12	46.35±171.08	18.23±14.55	0.47±1.38	0.01±0.01
	AA	4.4±1.28	2.51±2.15	2.47±0.83	1.21±0.61	34.99±64.33	18.56±18.2	0.65±1.26	0.01±0.01
	p	0.296	0.662	**0.038 ***	0.6	0.405	0.539	0.542	0.848

Notes: Data are presented as means SD or percentages; **p* value < 0.05 indicates statistical significance (*p* < 0.05) are in bold.
Abbreviations: HDL-C, high-density lipoprotein cholesterol; LDL-C, high-density lipoprotein cholesterol; TC, total cholesterol; ALBP, Adipocyte Lipid-binding Protein; INS, insulin; UCRP (ubiquitin cross-reacting protein); *KCNQ1*, Potassium Voltage-Gated Channel Subfamily Q Member 1; T2DM, type 2 diabetes mellitus.
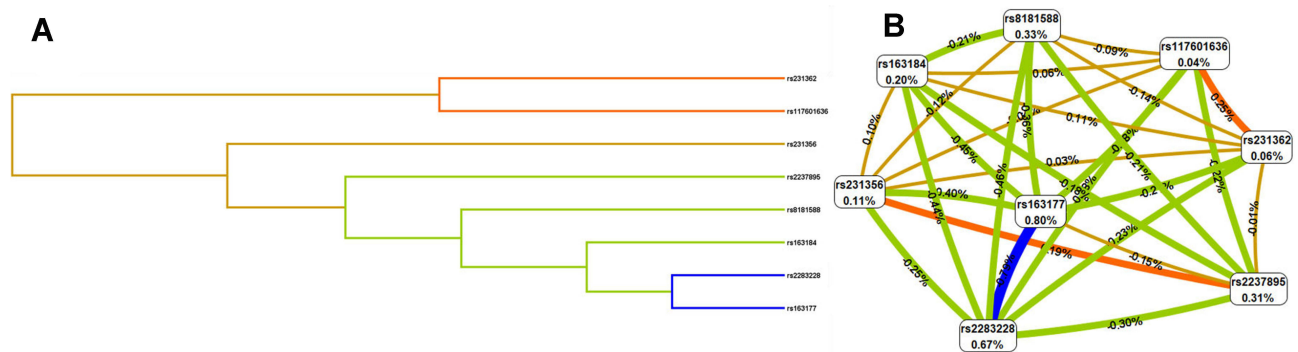
Figure 1 Dendrogram (**A**) and fruchterman Rheingold (**B**) of *KCNQ1* SNP-SNP interaction for T2DM risk. (**A**) Short connections among nodes represent stronger redundant interactions. (**B**) A Negative value for the two- locus entropy indicates that it is an antagonistic effect, and a positive value indicates that it is a synergistic effect.

different ethnic backgrounds to T2DM by altering insulin secretion.[25,26] For example, the rs2237895 allele was related to b-cell dysfunction in the Danish population.[8] The rs2283228 risk allele was associated with an elevated fasting glucose and impaired b-cell function in Asians.[27] In the present study, we selected eight common variants in *KCNQ1* to explore their correlations with T2DM risk. Currently, rs2283228 and rs2237895 have been widely reported in different populations, but their correlations with the risk of T2DM in different populations were inconsistent. Previous studies have shown that rs2237895 variant is associated with T2DM risk in Asians (Japanese, Chinese, and Koreans), and in Europeans (Danish and Scandinavian).[6,8,9,25,28,29] In contrast, rs2237895 were not associated with the incidence of T2DM in Punjabi and Asian Indian populations,[30] Singaporean (Chinese and Malays, Asian Indians) and Malaysian Chinese the

subjects, Spanish Renastur cohort or Tunisians.[27,31–33] Our research showd that rs2237895 and rs2283228 were significantly correlated with T2DM risk in the population of Northwestern China.

In addition, the association of rs163184 polymorphism to the earlier onset of T2DM has not been observed in the Slovakian population.[34] Our study found that rs163184 was associated with an increased risk of T2DM in the dominant model, which was inconsistent with the reports in the Slovakian population. Regarding the rs163177 locus, a study to identify SNPs associated with the risk of T2DM in Korean adults found a prospective association between rs163177 (*KCNQ1*) and T2DM.[35] We also found that this locus could increase the risk of T2DM in the population of Northwestern China, which was consistent with the results in the Korean population. The above studies in different population have both consistent and

Table 8 SNP–SNP Interaction Models of the *KCNQ1* Gene the Predisposition of T2DM

Model	Testing Bal. Acc.	CVC	OR (95% CI)	*p*
rs2283228	0.511	5/10	1.36 (1.06–1.75)	**0.017**
rs2237895, rs163177	0.509	8/10	1.63 (1.25–2.13)	**<0.001**
rs2237895, rs2283228, rs163177	0.524	5/10	1.92 (1.49–2.48)	**<0.001**
rs2237895, rs2283228, rs163177, rs8181588	0.546	9/10	2.62 (2.00–3.44)	**<0.001**
rs2237895, rs2283228, rs231356, rs163177, rs8181588	0.554	10/10	3.34 (2.54–4.40)	**<0.001**
rs2237895, rs2283228, rs231356, rs117601636, rs163177, rs8181588	0.568	10/10	4.10 (3.12–5.39)	**<0.001**
rs2237895, rs2283228, rs231356, rs231362, rs117601636, rs163177, rs8181588	0.555	10/10	4.75 (3.59–6.28)	**<0.001**
rs2237895, rs2283228, rs231356, rs231362, rs117601636, rs163177, rs163184, rs8181588	0.542	10/10	5.15 (3.85–6.90)	**<0.001**

Notes: *p* values were calculated using χ2 tests. Bold indicated that *p* < 0.05 meant the data was statistically significant.
Abbreviations: MDR, multifactor dimensionality reduction; Bal. Acc., balanced accuracy; CVC, cross–validation consistency; *KCNQ1*, Potassium Voltage-Gated Channel Subfamily Q Member 1; T2DM, type 2 diabetes mellitus.
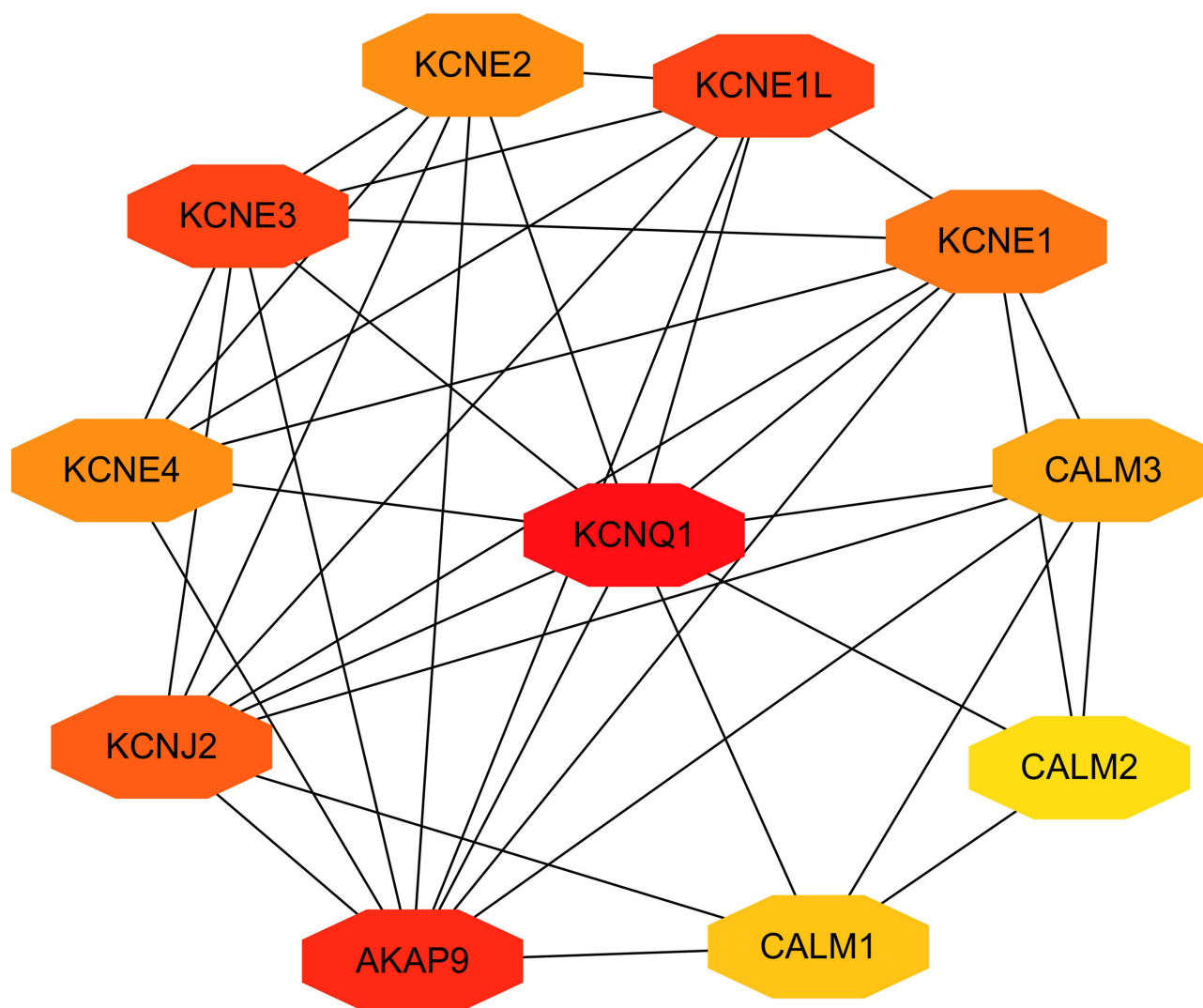
Figure 2 Clusters of screened PPI network. The deeper the color, the greater the degree of the node represent.

inconsistent places, which may be attributed to ethnic difference, environmental factor, or inappropriate sample size. At the same time, more sample studies are needed to confirm our results.

Tobacco smoking, an established modifier of DNA methylation, is associated with an increased risk of T2DM diabetes.[36] In a Dutch population-based cohort study, the allele of rs231356 was observed to be associated with hypomethylation of *KCNQ1* and a higher risk of diabetes.[37] In our study, we found that rs231356 was associated with the risk of T2DM neither in smokers nor in non-smokers, which may be due to the small sample size or ethnic differences and required more samples for further study.

The most common feature of classic patients with T2DM is obesity, but recent studies have shown that lean patients with T2DM exhibit more rapid, early loss of b-cell function while still having low levels of insulin resistance in contrast to obese patients with T2DM.[38] In our study, we found that four polymorphisms (rs231362 rs2283228, rs163184, and rs163177) were associated with an increased risk of diabetes at BMI $\leq$ 24, which further prove that the prevalence of lean T2DM patients was higher than that of obese T2DM patients.

T2DM is a complex disease affected by the interaction of multiple factors. Multi-gene or SNP-SNP interaction
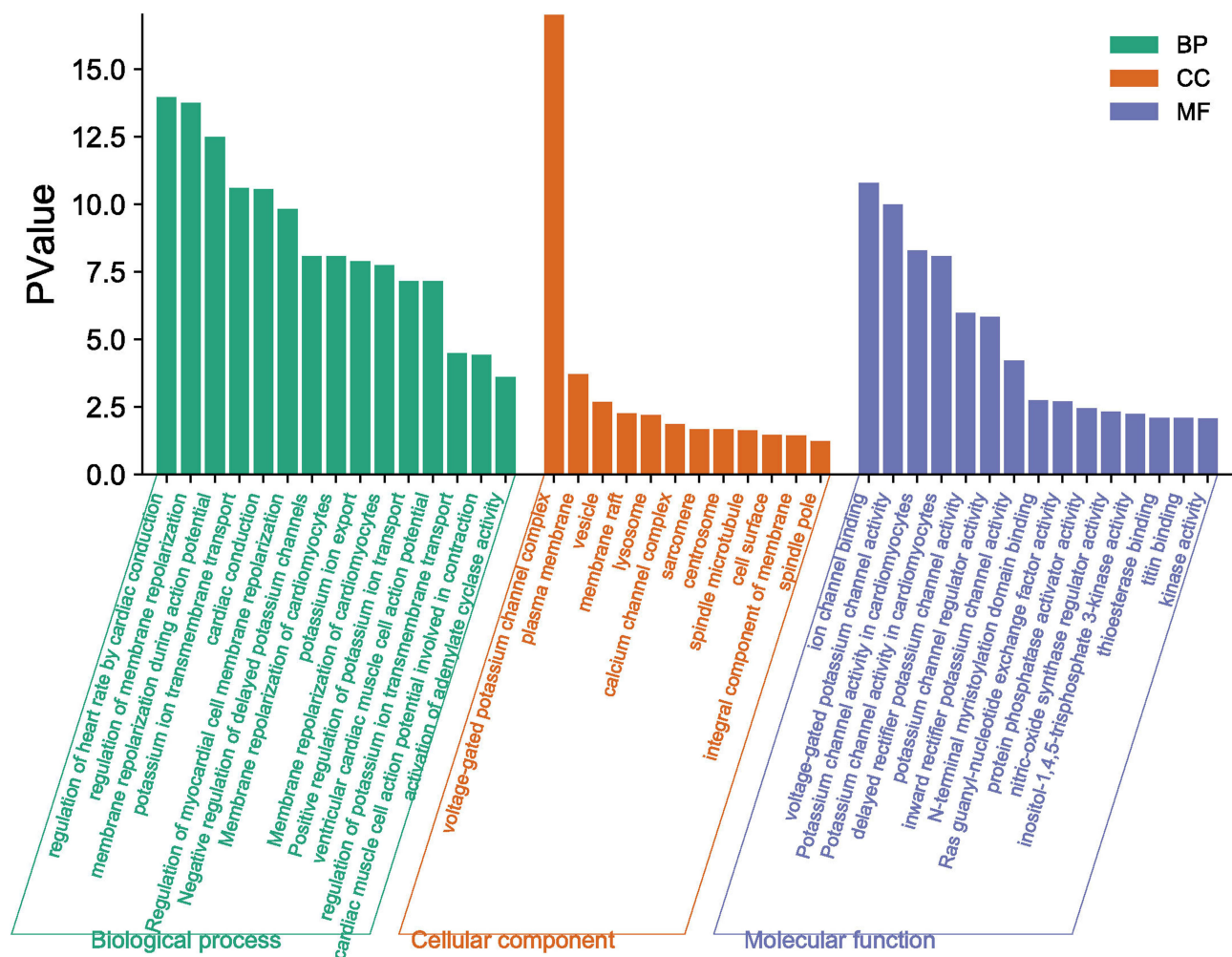
Figure 3 GO enrichment analysis.

analysis is helpful to discover the risk factors of T2DM. Therefore, we perform MDR analysis to determine the potential SNP-SNP interactions among the 8 SNPs in the *KCNQ1* gene. The results show that rs2237895, rs2283228, rs231356, rs117601636, rs163177, and rs8181588 each act as the best model for the contribution of T2DM susceptibility, and increase the risk of T2DM.

Some limitations in our study must be noted. This study only initially explored the correlation between *KCNQ1* polymorphism and T2DM in Northwestern China. The specific mechanism of *KCNQ1*'s role in T2DM is still unclear. In the future, we will further

verify our results in large samples, and conduct cell and animal experiments to explore the specific mechanism of *KCNQ1* on T2DM.

Conclusion

This study reported for the first time that *KCNQ1* polymorphism is associated with the risk of T2DM in Northwestern Chinese population. This result may enrich the research between *KCNQ1* and T2DM and provide a scientific basis for early screening, prevention, and diagnosis of T2DM high-risk populations in Northwest China.
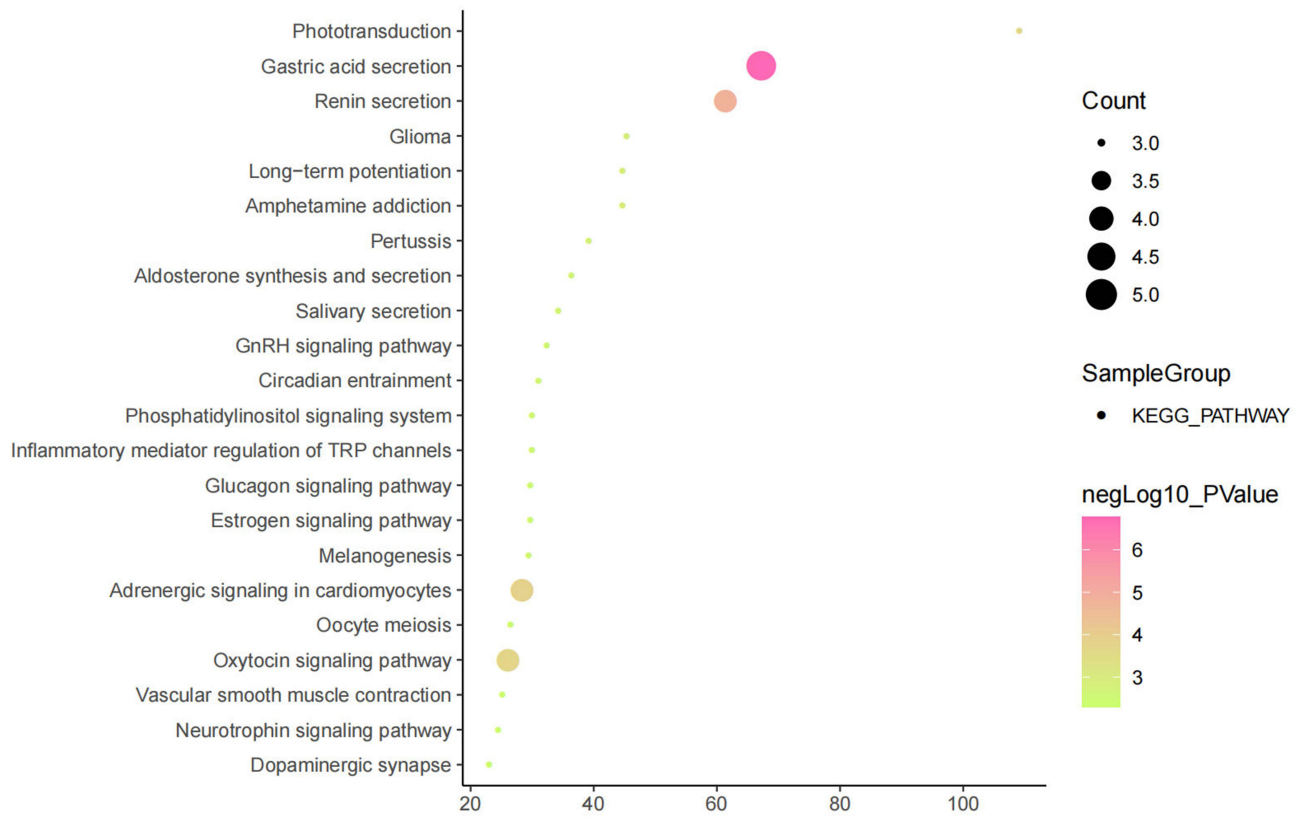
Figure 4 Results of KEGG analysis.
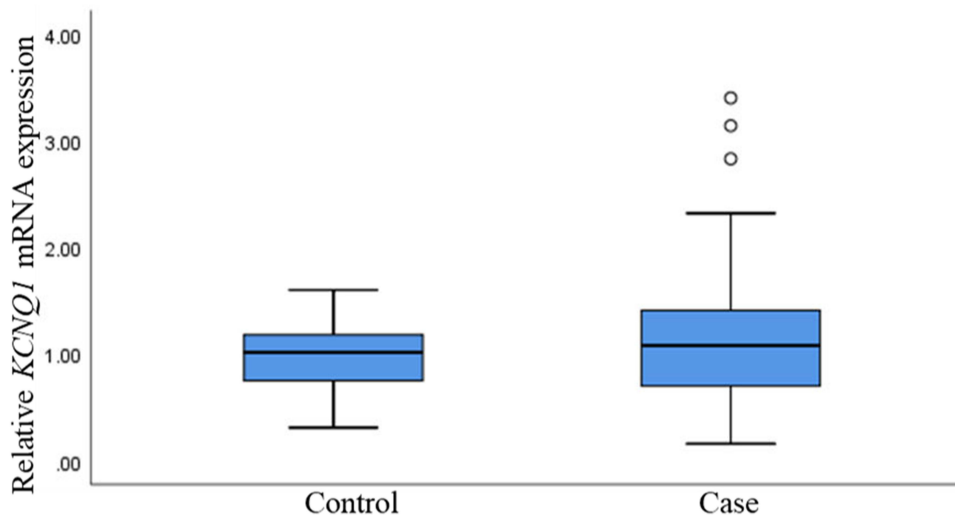
Figure 5 Relative mRNA expression of *KCNQ1* genes in T2DM and controls. T2DM, type 2 diabetes mellitus.
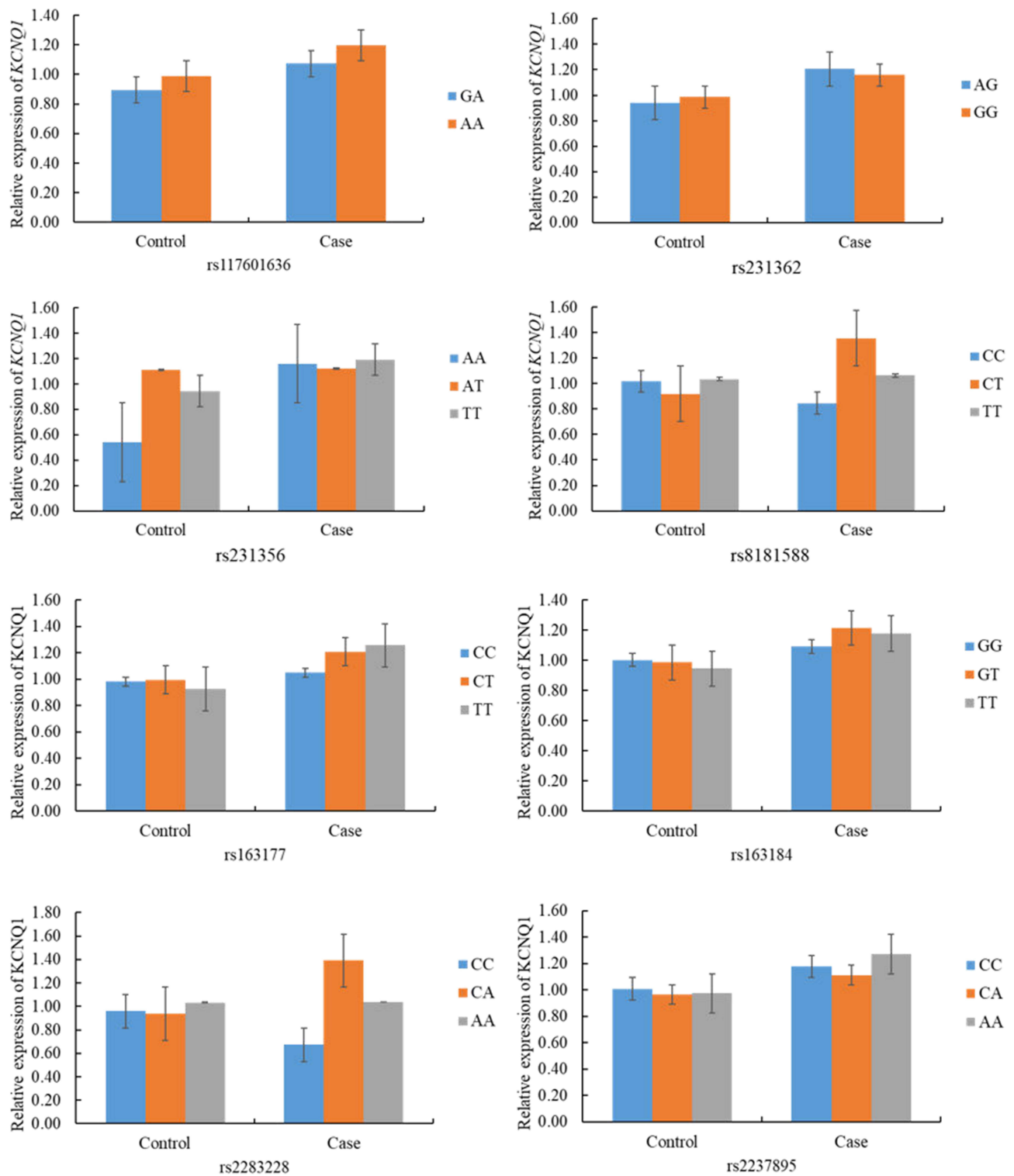
Figure 6 Association of *KCNQ1* relative mRNA expression and genetic polymorphisms in T2DM and controls.

Data Sharing Statement

The datasets used or analyzed during the current study are available from the corresponding author on reasonable request.

Ethics Statement

This study was approved by the Ethics Committee of the First Affiliated Hospital of Xi'an Jiaotong University, and in compliance with the Declaration of Helsinki. The purpose of this study was well informed to the all participants and written informed consents were obtained from all participants prior to biological material collection in this study. All subsequent study analyses were conducted in accordance with the approved guidelines and regulations.

Consent for Publication

Written informed consent was obtained from the patient for publication of this report.

Author Contributions

BYS and WC: conceived and designed the experiments;

JX, WZ and WS: performed the experiments;

JX and JQC: analyzed the data;

YNT, HC and PH: contributed reagents/materials/analysis tools;

SJY and LW: prepared the figures and/or tables;

XH and LW: drafted the work or revised it critically for important content.

All authors have read and approved the manuscript. All authors contributed to data analysis, drafting or revising the article, have agreed on the journal to which the article will be submitted, gave final approval of the version to be published, and agree to be accountable for all aspects of the work.

Disclosure

The authors declare that they have no conflict of interests.

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