Research Article

A Novel Polymorphism (rs35612982) in *CDKAL1* Is a Risk Factor of Type 2 Diabetes: A Case-Control Study

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Keywords

Type 2 diabetes · *CDKAL1* gene · Polymorphisms · Case-control

Abstract

Background: The interaction of environmental factors and genetic factors may contribute to the risk of type 2 diabetes (T2D). We aimed to investigate whether age, gender, body mass index (BMI) and lifestyle factors have an effect on the association between the *CDKAL1* polymorphisms and T2D. **Methods:** Eight single nucleotide polymorphisms in *CDKAL1* were genotyped by Agena MassARRAY in 508 T2D patients and 503 controls. The association between the *CDKAL1* polymorphisms and T2D was evaluated using logistic regression model by calculating OR and 95% CIs. **Results:** We found a significant association between *CDKAL1* polymorphisms (rs4712523, OR 1.42, $p = 9.44 \times 10^{-5}$; rs4712524, OR 1.38, $p = 3.28 \times 10^{-4}$; rs10946398, OR 1.43, $p = 6.21 \times 10^{-5}$; rs7754840, OR 1.43, $p = 6.33 \times 10^{-5}$; rs35612982, OR 1.34, $p = 0.0010$; and rs10440833, OR 1.32, $p = 0.0018$) and T2D risk among the Han population from Northwest China. We also found that genetic variants of *CDKAL1* could modify the risk of T2D that might be influenced by age, BMI and the status of smoking and drinking. Besides, rs35612982-CT ($p =$

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0.038) and rs10440833-AT ($p = 0.044$) genotypes were higher insulin level. **Conclusion:** *CDKAL1* rs35612982 (C/T) polymorphism, as a new polymorphism, was associated with the increased risk of T2D in the Han Chinese population. Moreover, the contribution of *CDKAL1* polymorphisms to T2D risk seems to be associated with age, gender, BMI, smoking and drinking.

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Background

Type 2 diabetes (T2D) is a serious and common chronic metabolic disease characterized by insufficient insulin secretion leading to hyperglycaemia, and is a major life-threatening health problem throughout the world [1]. According to the World Health Organization in 2014, diabetes prevalence reached 422 million, with T2D accounting for more than 90% of these cases [2]. In China, the incidence of T2D in adults has been increasing over recent decades [3]. T2D is a complex, heterogeneous and multifactorial disorder caused by genetic and environmental factors such as obesity and unhealthy lifestyle [4]. Genetic susceptibility appears to have a crucial role in the aetiology and manifestation of the disease. Genome-wide association studies have pointed that some single nucleotide polymorphisms (SNPs) are important risk factors for T2D, which is associated with the mortality among diabetic patients [5, 6].

CDKAL1 gene, mapped to chromosome 6p22.3, encodes cyclin-dependent kinase 5 regulatory subunit-associated protein 1 (CDK5RAP1)-like 1. CDK5 is a serine/threonine protein kinase, which contributed to the glucose-dependent regulation of the insulin secretion. CDK5 plays a permissive role in the glucotoxicity, the pathophysiology of β -cell dysfunction and predisposition to T2D [7]. *CDKAL1* has the protein domain similarity with CDK5RAP1, which is a negative regulator of CDK5 through the inhibition of the CDK5 activator p35 [8]. *CDKAL1* expression in human pancreatic beta-cells [9] supports the correlation of CDKAL1- and CDK5-mediated pathways. Consistently, several studies showed the association of genetic variants in *CDKAL1* with the defects in proinsulin conversion and insulin response upon glucose stimulation [10, 11]. Thus, *CDKAL1* is a well-accepted pathogenesis-related key protein for T2D.

Genome-wide association studies have identified several SNPs in the *CDKAL1* gene associated with T2D [12, 13]. A recent study has reported the significant association between *CDKAL1* variants and susceptibility to T2D in Caucasian and Asian but not African populations [14], indicating that the association is an ethnic difference. This necessitates the replicating the association of these variants with T2D risk in different ethnicities. Furthermore, whether there is the contribution of non-genetic factors in the association between *CDKAL1* SNPs and T2D needs to be investigated.

Here, we conducted a case-control study to examine the effects of age, gender, body mass index (BMI), smoking and alcohol consumption, and *CDKAL1* polymorphisms on the risk of T2D, and to explore the association of *CDKAL1* SNPs with clinical characteristics among T2D patients in the Han population from Northwest China. Especially, there are no published reports assessing the role of *CDKAL1* rs35612982 and rs10440833 variants in the susceptibility of T2D in a Chinese Han population.

Methods

Study Participants

A total of 508 T2D patients and 503 age- and gender-matched healthy controls with normal blood glucose levels were recruited from the First Affiliated Hospital of Xi'an Jiaotong University in this study. All recruited subjects were unrelated ethnic Han Chinese. All patients

were newly diagnosed with T2D based on fasting plasma glucose ≥ 7.0 mmol/L and/or post-prandial plasma glucose ≥ 11.1 mmol/L, in keeping with the WHO criteria [15]. Patients with type 1 diabetes mellitus, gestational diabetes, acute or chronic diseases of the liver, kidney, or heart or other endocrine disease, inflammatory diseases, or malignant tumours were excluded. Healthy individuals with a normal fasting glucose level and without history of T2D and other chronic diseases were recruited as controls. This case-control study was carried out with the approval of the Ethics Committee of the First Affiliated Hospital of Xi'an Jiaotong University and in accordance with the guidelines of the Declaration of Helsinki. All participants were informed about the purpose of this study and signed a written informed consent.

DNA Isolation and Genotyping

The peripheral blood (5 mL) of all the subjects was collected in EDTA-coating tubes. The epidemiological and clinical data were collected with a questionnaire and medical records. The following data were recorded: age, gender, BMI (kg/m^2), smoking habits, alcohol consumption, and clinical parameters. Genomic DNA was isolated from peripheral blood leukocytes by GoldMag DNA Purification Kit (GoldMag Co. Ltd., Xi'an, China), quantified by NanoDrop 2000 (Thermo Scientific, Waltham, MA, USA), then stored at -20°C for further analysis.

Eight *CDKAL1* SNPs (rs4712523, rs4712524, rs10946398, rs7754840, rs7756992, rs35612982, rs10440833 and rs6931514) were selected based on diabetes-related polymorphisms reported by previous GWAS studies, the NCBI SNP database (<https://www.ncbi.nlm.nih.gov/snp/>), and minor allele frequencies $>5\%$ in the 1000 Genomes Project data (<http://www.internationalgenome.org/>). The potential function *CDKAL1* polymorphisms were estimated by HaploReg version 4.1 (<https://pubs.broadinstitute.org/mammals/haploreg/haploreg.php>). Genotyping was performed using Agena MassARRAY system (Agena, San Diego, CA, USA) according to the manufacturer's instructions [16, 17]. The primers for PCR amplification and single base extension were shown in online supplementary Table 1 (for all online suppl. material, see www.karger.com/doi/10.1159/000503175). Cases and controls were randomized during genotyping. Approximately 5% of the samples were genotyped in duplicate, and the concordance was 100%.

Statistical Analysis

All data were analyzed by SPSS version 18.0 (SPSS Inc., Chicago, IL, USA) and PLINK software. Student *t* test or chi-square test was used to compare the difference of characteristics between T2D patients and healthy controls. Demographic and clinical variables were reported as mean (SD) for continuous variables and frequency (percentage) for categorical variables. Hardy-Weinberg equilibrium in controls was determined via a goodness-of-fit χ^2 test. ORs and 95% CIs for multiple inheritance models (allelic, genotype, dominant, recessive and log-additive) were calculated by χ^2 and logistic regression analysis to estimate the relationship between *CDKAL1* polymorphisms and T2D susceptibility. Bonferroni method was used to correct for multiple comparisons in the overall subjects. Pairwise linkage disequilibrium (LD) and haplotype analyses were analyzed using Haploview (version 4.2) and PLINK software. A two-tailed *p* value <0.05 was considered statistically significant, whereas a value of corrected *p* $<0.05/(8 \times 5)$ was considered significant after Bonferroni correction.

Meta-Analysis

A comprehensive search in PubMed, Embase, and the China National Knowledge Infrastructure databases was conducted using the following search terms: "*CDKAL1*," "rs10946398," "rs7754840" and "T2D" up to March of 2019. The articles selected for the subsequent meta-analysis were all published in English in the primary literature. For rs10946398 [18–30] and

Table 1. Characteristics of patients with T2D and controls

Variable	Cases (n = 508)	Controls (n = 503)	p value
Age, years, mean (SD), n (%)	59.21 (11.90)	59.34 (7.62)	0.841
>59	263 (51.8)	265 (52.7)	
≤59	245 (48.2)	238 (47.3)	
Gender, male/female, n (%)			0.712
Male	277 (54.5)	279 (55.5)	
Female	231 (45.5)	224 (44.5)	
BMI, kg/m ² , n (%)			
<24	130 (25.6)	173 (34.4)	
≥24	187 (36.8)	185 (36.8)	
Unavailable	191 (37.6)	145 (28.8)	
Smoking, n (%)			
Yes	135 (26.6)	115 (22.9)	
No	230 (45.3)	188 (37.4)	
Unavailable	143 (28.1)	200 (39.8)	
Drinking, n (%)			
Yes	68 (13.4)	106 (21.1)	
No	277 (54.5)	182 (36.2)	
Unavailable	163 (32.1)	215 (42.7)	
Age at diagnosis, years, mean (SD)	49.05 (12.20)		
T2D with retinopathy, n (%)			
Yes	148 (29.1)		
No	182 (35.8)		
Unavailable	178 (35.0)		
Diabetes duration, years, n (%)			
>9	150 (29.5)		
≤9	185 (36.4)		
Unavailable	173 (34.1)		
Total cholesterol, mmol/L	4.61 (0.88)	4.30 (1.63)	0.029
HDL-C, mmol/L	2.59 (0.75)	2.49 (1.16)	0.172
LDL-C, mmol/L	1.12 (0.25)	1.50 (7.45)	0.378
Serum uric acid, μmol/L	6.80 (19.8)	5.96 (3.38)	0.396
Creatinine, μmol/L	67.85 (32.08)	65.86 (32.18)	0.390
GFR, mL/min	95.95 (13.11)	122.61 (35.88)	<0.001
Fasting blood glucose	9.95 (4.70)		
Glycated hemoglobin	9.30 (2.48)		
Triglyceride	2.50 (2.26)		
Urea	6.25 (3.19)		
Creatinine	68.97 (29.49)		
Cystatin C	0.97 (2.18)		
ALBP	27.66 (52.01)		
GFR	122.61 (35.88)		
CRP	1.38 (1.57)		
INS	18.80 (18.65)		
25QWD	24.69 (15.37)		
UCRP	0.54 (1.28)		
RBP	38.75 (11.14)		

Bold indicate that $p < 0.05$ indicates statistical significance.

T2D, type 2 diabetes; BMI, body mass index; HDL-C, high-density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol; ALBP, adipocyte lipid-binding protein; GFR, glomerular filtration rate; CRP, C-reactive protein; INS, insulin; 25QWD, 25 hydroxy-vitamin D; UCRP, ubiquitin cross-reactive protein; RBP, retinol-binding protein.

Table 2. Relationships between the susceptibility to T2D and confounding factors

Variable	OR	95% CI	p value
Age (>59 vs. ≤59 years)	0.70	0.48–1.01	0.056
Gender (male vs. female)	1.19	0.78–1.81	0.427
BMI (>24 vs. ≤24 kg/m ²)	1.57	1.09–2.25	0.014
Smoking status (yes vs. no)	1.49	0.90–2.47	0.122
Drinking status (yes vs. no)	0.25	0.15–0.42	<0.001

Bold values indicate that $p < 0.05$ indicates statistical significance. p values were calculated by logistic regression analysis with adjustments for age and gender. T2D, type 2 diabetes; BMI, body mass index.

rs7754840 [20, 22, 29, 31–39], we found 13 and 12 articles respectively. STATA version 11.0 (Stata Corporation, TX, USA) was used to analysis the association of *CDKAL1* 10946398 and rs7754840 variants with the risk of T2D. The pooled OR in the allele model was assessed by the Z-test, and $p < 0.05$ was considered statistical significance. The pooled OR was calculated by a random-effects model if $I^2 > 50\%$ or $P_Q < 0.1$, which indicated heterogeneity was significant; otherwise, the pooled OR was assessed by a fixed-effects model [40].

Results

A total of 508 T2D patients (277 males and 231 females, mean age 59.21 [11.90]) and 214 healthy controls (279 males and 224 females, mean age 59.34 [7.62]) were recruited. Table 1 displayed the clinical and biochemical characteristics of the participants in detail. There was no significant difference in the distribution of gender and age between T2D patients and the healthy controls ($p = 0.841$ and 0.712 respectively). The level of total cholesterol ($p = 0.029$) and GFR ($p < 0.001$) differed significantly between the T2D group and the normoglycaemic health control group.

Logistic regression analysis was used to assess the relationship between the susceptibility of T2D and confounding factors. We found that BMI >24 kg/m² (OR 1.57, 95% CI 1.09–2.25, $p = 0.014$) was significantly associated with a higher risk for T2D, while drinking (OR 0.25, 95% CI 0.15–0.42, $p < 0.001$) showed a significant negative association with T2D (Table 2).

The detailed information of candidate SNPs in *CDKAL1* was listed in Table 3. The minor allele frequencies of these SNPs were $>5\%$, and all SNPs were in accordance with Hardy-Weinberg equilibrium among the controls ($p > 0.05$). Results of allele model showed that 6 SNPs in *CDKAL1* were significantly associated with increased T2D risk with overall effects (OR) from 1.34 to 1.43 (rs4712523, OR 1.42, 95% CI 1.19–1.69, $p = 9.44 \times 10^{-5}$; rs4712524, OR 1.38, 95% CI 1.16–1.65, $p = 3.28 \times 10^{-4}$; rs10946398, OR 1.43, 95% CI 1.20–1.71, $p = 6.21 \times 10^{-5}$; rs7754840, OR 1.43, 95% CI 1.20–1.71, $p = 6.33 \times 10^{-5}$; rs35612982, OR 1.34, 95% CI 1.12–1.60, $p = 0.0010$; and rs10440833, OR 1.32, 95% CI 1.11–1.58, $p = 0.0018$). Considering multiple comparisons, we used Bonferroni correction on the results and $p < 0.05/(8 \times 5)$ was considered significant threshold. The significance between *CDKAL1* polymorphisms (rs4712523, rs4712524, rs10946398, rs7754840, and rs35612982) still existed after Bonferroni correction.

The genotype frequencies of *CDKAL1* polymorphisms were shown in Table 4 and online supplementary Table 2. Among these variants, the genotype frequencies containing “two” risk alleles of rs4712523, rs4712524, rs10946398 and rs7754840 variants were significantly different between case and control groups ($p < 0.05$). After Bonferroni correction, rs4712523

Table 3. The information about the candidate SNPs in *CDKAL1* and associations with the risk of T2D in the allele model

SNP ID	Chr: position	Alleles (minor/ major)	Frequency (MAF)		<i>p</i> value for HWE	OR (95% CI)	<i>p</i> value	HaploReg
			case	control				
rs4712523	6:20657333	G/A	0.502	0.416	0.582	1.42 (1.19–1.69)	9.44 × 10⁻⁵	Promoter histone marks, Enhancer histone marks, DNase, proteins bound, Motifs changed
rs4712524	6:20657634	G/A	0.502	0.422	0.268	1.38 (1.16–1.65)	3.28 × 10⁻⁴	Promoter histone marks, Enhancer histone marks, DNase, Motifs changed
rs10946398	6:20660803	C/A	0.499	0.410	0.783	1.43 (1.20–1.71)	6.21 × 10⁻⁵	DNase, Motifs changed
rs7754840	6:20661019	C/G	0.498	0.410	0.782	1.43 (1.20–1.71)	6.33 × 10⁻⁵	Promoter histone marks, Enhancer histone marks, DNase, Motifs changed
rs7756992	6:20679478	A/G	0.451	0.487	0.788	0.87 (0.73–1.03)	0.1045	Enhancer histone marks, Motifs changed, GRASP QTL hits
rs35612982	6:20682391	C/T	0.475	0.403	0.578	1.34 (1.12–1.60)	0.0010	Enhancer histone marks, Motifs changed
rs10440833	6:20687890	A/T	0.477	0.409	0.854	1.32 (1.11–1.58)	0.0018	Motifs changed
rs6931514	6:20703721	A/G	0.453	0.489	0.929	0.87 (0.73–1.03)	0.1034	Enhancer histone marks, Motifs changed

Bold values indicate that after Bonferroni correction ($p < 0.05/[8 \times 5]$) means the data is statistically significant.

SNP, single nucleotide polymorphism; T2D, type 2 diabetes; MAF, minor allele frequency; HWE, Hardy-Weinberg equilibrium.

and rs4712524 polymorphisms were associated with the significant susceptibility to T2D under homozygote and additive models (rs4712523: OR 1.97, $p = 1.49 \times 10^{-4}$ and OR 1.40, $p = 1.43 \times 10^{-4}$; rs4712524, OR 1.85, $p = 5.85 \times 10^{-4}$ and OR 1.36, $p = 4.85 \times 10^{-4}$; Table 4). Rs10946398 (homozygote: OR 2.03, $p = 8.89 \times 10^{-5}$; recessive: OR 1.67, $p = 9.91 \times 10^{-4}$; and additive: OR 1.42, $p = 9.04 \times 10^{-5}$) and rs7754840 (homozygote: OR 2.03, $p = 9.07 \times 10^{-5}$; recessive: OR 1.68, $p = 9.37 \times 10^{-4}$; and additive: OR 1.42, $p = 9.39 \times 10^{-5}$) variants had a higher risk for T2D after Bonferroni correction. In addition, rs35612982 (OR 1.78, $p = 0.0014$; OR 1.40, $p = 0.0121$; OR 1.54, $p = 0.0062$ and OR 1.33, $p = 0.0015$) and rs10440833 (OR 1.75, $p = 0.0020$; OR 1.38, $p = 0.0190$; OR 1.53, $p = 0.0070$ and OR 1.31, $p = 0.0022$). SNPs also contributed to the increased T2D risk under multiple genetic models, while the significance was lost when Bonferroni correction was conducted. However, there was no statistically significant association between other 2 SNPs (rs7756992 and rs6931514) and T2D risk ($p > 0.05$, online suppl. Table 2).

We next conducted LD and haplotype analyses of *CDKAL1* polymorphisms. Figure 1 revealed the existence of 2 LD blocks in *CDKAL1* SNPs (Block 1: rs4712523, rs4712524, rs10946398 and rs7754840; Block 2, rs7756992, rs35612982, rs10440833 and rs6931514). The frequencies of haplotypes in the case and control groups were presented in Table 5. The haplotypes “GGCC” (OR 1.42, $p = 9.39 \times 10^{-5}$) and “AAAG” (OR 1.36, $p = 5.33 \times 10^{-4}$) in block 1 and “ATTA” (OR 1.33, $p = 0.0013$) and “GCAG” (OR 1.55, $p = 0.0084$) in block 2 were significantly associated with the higher risk of T2D after being adjusted by age and gender.

We further analyzed whether the genotypic effects on T2D risk were dependent on gender and age (online suppl. Table 3). We found that rs4712523, rs4712524, rs10946398, rs7754840, rs35612982 and rs10440833 variants were associated with an increased risk of T2D in both males and females ($p < 0.05$). Rs4712523, rs10946398, and rs7754840 polymorphisms were at higher risk of developing T2D in subjects at age >59 years or at age ≤59 years ($p < 0.05$). Interestingly, associations between *CDKAL1* SNPs (rs4712524, rs35612982 and rs10440833) and the increased risk of T2D were found only among participants younger than 59 years of age.

We further examined whether BMI, smoking and drinking status had an effect on the association of *CDKAL1* polymorphisms with T2D risk. As shown in online supplementary Table 4, we also found that rs4712523, rs4712524, rs10946398, rs7754840, rs35612982 and rs10440833 variants had increased risk of developing T2D in subgroup with BMI >24 kg/m², non-smokers and non-drinking ($p < 0.05$). Moreover, the significant associations between

Table 4. Relationships between *CDKAL1* polymorphisms and T2D risk

SNP ID	Model	Genotype	Case	Control	Adjusted by age and gender	
					OR (95% CI)	p value
rs4712523	Genotype	AA	131	175	1.00	
		AG	246	238	1.38 (1.03–1.84)	0.0291
		GG	133	90	1.97 (1.39–2.81)	1.49 × 10⁻⁴
	Dominant	AA	131	175	1.00	
		AG-GG	379	328	1.54 (1.18–2.02)	0.0017
	Recessive	AA-AG	377	413	1.00	
		GG	133	90	1.62 (1.20–2.19)	0.0018
	Log-additive	–	–	–	1.40 (1.18–1.67)	1.43 × 10⁻⁴
rs4712524	Genotype	AA	130	171	1.00	
		AG	246	228	1.42 (1.06–1.90)	0.0189
		GG	132	94	1.85 (1.30–2.62)	5.85 × 10⁻⁴
	Dominant	AA	130	171	1.00	
		AG-GG	378	322	1.54 (1.17–2.03)	0.0018
	Recessive	AA-AG	376	399	1.00	
		GG	132	94	1.49 (1.11–2.01)	0.0090
	Log-additive	–	–	–	1.36 (1.15–1.62)	4.85 × 10⁻⁴
rs10946398	Genotype	AA	132	176	1.00	
		AC	247	240	1.37 (1.03–1.83)	0.0319
		CC	131	86	2.03 (1.43–2.89)	8.89 × 10⁻⁵
	Dominant	AA	132	176	1.00	
		AC-CC	378	326	1.54 (1.18–2.02)	0.0017
	Recessive	AA-AC	379	416	1.00	
		CC	131	86	1.67 (1.23–2.27)	9.91 × 10⁻⁴
	Log-additive	–	–	–	1.42 (1.19–1.69)	9.04 × 10⁻⁵
rs7754840	Genotype	GG	133	177	1.00	
		GC	246	240	1.36 (1.02–1.82)	0.0349
		CC	131	86	2.03 (1.42–2.89)	9.07 × 10⁻⁵
	Dominant	GG	133	177	1.00	
		GC-CC	377	326	1.54 (1.17–2.01)	0.0018
	Recessive	GG-GC	379	417	1.00	
		CC	131	86	1.68 (1.23–2.28)	9.37 × 10⁻⁴
	Log-additive	–	–	–	1.42 (1.19–1.69)	9.39 × 10⁻⁵
rs35612982	Genotype	TT	147	182	1.00	
		TC	241	235	1.27 (0.96–1.68)	0.0981
		CC	122	85	1.78 (1.25–2.53)	0.0014
	Dominant	TT	147	182	1.00	
		TC-CC	363	320	1.40 (1.08–1.83)	0.0121
	Recessive	TT-TC	388	417	1.00	
		CC	122	85	1.54 (1.13–2.10)	0.0062
	Log-additive	–	–	–	1.33 (1.11–1.58)	0.0015

Table 4 (continued)

SNP ID	Model	Genotype	Case	Control	Adjusted by age and gender	
					OR (95% CI)	p value
rs10440833	Genotype	TT	144	177	1.00	
		TA	244	241	1.24 (0.94–1.65)	0.1306
		AA	121	85	1.75 (1.23–2.49)	0.0020
	Dominant	TT	144	177	1.00	0.0190
		TA-AA	365	326	1.38 (1.05–1.79)	
	Recessive	TT-TA	388	418	1.00	0.0070
		AA	121	85	1.53 (1.12–2.09)	
	Log-additive	–	–	–	1.31 (1.10–1.56)	0.0022

Bold values indicate that after Bonferroni correction ($p < 0.05/[8 \times 5]$) means the data is statistically significant.
p values were calculated by logistic regression analysis with adjustments for age and gender.
SNP, single nucleotide polymorphism; T2D, type 2 diabetes.

Fig. 1. Haplotype block map for 8 SNPs in the *CDKAL1* gene.

rs35612982 and rs6931514 polymorphisms and T2D susceptibility were observed only in non-smokers ($p < 0.05$).

To evaluate the effect of diabetes duration and retinopathy, T2D patients were divided into 2 groups according to diabetes duration and retinopathy (online suppl. Table 5). Rs4712523 (OR 1.84, $p = 0.0281$), rs10946398 (OR 1.81, $p = 0.0332$) and rs7754840 (OR 1.81, $p = 0.0332$) variants in *CDKAL1* were found to be associated with the increased risk of T2D with retinopathy. However, no significant correlation was found in diabetes duration.

The relation between the genotypes of *CDKAL1* SNPs and the biochemical indexes in T2D patients was investigated. As shown in Table 6 and online supplementary Table 6, the urea levels for different genotypes of rs7756992 ($p = 0.003$), rs35612982 ($p = 0.015$), rs10440833 ($p =$

Table 5. Haplotype frequencies in *CDKAL1* and their associations with T2D risk

Block	SNPs	Haplotype	Frequency		Crude analysis		Adjusted by age and gender	
			case	control	OR (95% CI)	p value	OR (95% CI)	p value
Block 1	rs4712523 rs4712524 rs10946398 rs7754840	GGCC	0.498	0.410	1.42 (1.19-1.69)	8.96×10^{-5}	1.42 (1.19-1.69)	9.39×10^{-5}
	rs4712523 rs4712524 rs10946398 rs7754840	AAAG	0.503	0.424	1.36 (1.14-1.61)	5.10×10^{-4}	1.36 (1.14-1.61)	5.33×10^{-4}
Block 2	rs7756992 rs35612982 rs10440833 rs6931514	ATTA	0.550	0.515	1.15 (0.97-1.37)	0.1185	1.15 (0.96-1.36)	0.1203
	rs7756992 rs35612982 rs10440833 rs6931514	GCAG	0.473	0.399	1.33 (1.12-1.58)	0.0012	1.33 (1.12-1.58)	0.0013
	rs7756992 rs35612982 rs10440833 rs6931514	GTTG	0.930	0.898	1.55 (1.12-2.15)	0.0085	1.55 (1.12-2.15)	0.0084

Bold values indicate that $p < 0.05$ indicates statistical significance.

The block 1 comprises the 4 closely linked SNPs rs4712523, rs4712524, rs10946398 and rs7754840. The block 2 comprises the 4 closely linked SNPs rs7756992, rs35612982, rs10440833 and rs6931514. p values were calculated using logistic regression analysis with and without adjustment by gender and age. SNP, single nucleotide polymorphism; T2D, type 2 diabetes.

Fig. 2. Forest plot of *CDKAL1* rs7754840 polymorphism and T2D susceptibility (allele model). The horizontal lines represent the study-specific ORs and 95% CIs. The diamond represents the pooled results of OR and 95% CI.

0.015) and rs6931514 ($p = 0.005$) had a significant difference. A significant association was observed between *CDKAL1* rs7756992 and rs6931514 genotypes and the levels of creatinine ($p = 0.045$ and $p = 0.037$, respectively). Besides, rs35612982-CT ($p = 0.038$) and rs10440833-AT ($p = 0.044$) genotypes had a higher insulin level. However, there was no difference in the remaining lipid parameters among the genotypes of the selected SNPs ($p > 0.05$ for all).

Table 6. Comparisons of clinical characteristics among T2D patients with different genotypes of *CDKAL1* SNPs

Characteristics	rs7756992				rs35612982			
	AA	AG	GG	p value	TT	CT	CC	p value
Fasting blood glucose	10.01 (3.76)	9.52 (3.98)	10.57 (6.04)	0.221	10.02 (3.51)	9.88 (5.05)	10.03 (5.22)	0.964
Glycated haemoglobin	9.54 (2.57)	9.13 (2.02)	9.39 (2.98)	0.496	9.52 (2.48)	9.23 (2.09)	9.16 (3.10)	0.593
Total cholesterol	4.51 (1.43)	4.58 (1.28)	4.50 (1.31)	0.868	4.48 (1.38)	4.57 (1.26)	4.53 (1.36)	0.853
Triglyceride	2.43 (2.18)	2.38 (1.87)	2.70 (2.77)	0.545	2.43 (2.15)	2.54 (2.28)	2.48 (2.39)	0.936
LDL-C	2.60 (1.00)	2.74 (1.12)	2.47 (0.93)	0.068	2.62 (0.95)	2.71 (1.11)	2.46 (0.99)	0.160
HDL-C	1.20 (0.60)	1.23 (0.61)	1.17 (0.52)	0.687	1.25 (0.66)	1.18 (0.60)	1.18 (0.42)	0.619
Urea	7.27 (5.55)	5.86 (1.80)	6.12 (2.48)	0.003	6.97 (4.93)	6.03 (2.06)	5.83 (2.23)	0.015
Creatinine	71.47 (31.22)	65.12 (17.43)	72.97 (39.49)	0.045	69.83 (28.60)	67.26 (29.29)	71.14 (30.87)	0.529
Cystatin C	1.47 (4.70)	0.82 (0.21)	0.88 (0.68)	0.136	1.29 (3.99)	0.82 (0.22)	0.88 (0.77)	0.267
ALBP	20.24 (43.46)	31.67 (57.38)	26.52 (48.50)	0.411	29.18 (56.25)	31.32 (58.73)	18.45 (24.75)	0.275
GFR	116.45 (37.76)	125.09 (35.63)	122.10 (34.25)	0.300	119.40 (37.95)	123.45 (36.9)	124.7 (31.63)	0.612
CRP	1.29 (0.76)	1.53 (2.07)	1.18 (0.91)	0.234	1.30 (0.85)	1.50 (2.08)	1.23 (0.99)	0.433
INS	15.76 (13.08)	21.26 (23.50)	16.91 (12.01)	0.090	16.12 (12.64)	21.86 (24.07)	16.27 (10.63)	0.038
25QWD	27.30 (20.01)	24.60 (16.90)	23.02 (6.86)	0.344	26.08 (17.89)	24.33 (16.95)	23.71 (6.69)	0.672
UCRP	0.60 (1.35)	0.48 (1.03)	0.59 (1.53)	0.734	0.61 (1.26)	0.58 (1.50)	0.39 (0.70)	0.506
RBP	39.41 (10.59)	39.70 (11.71)	36.99 (10.6)	0.259	39.58 (10.61)	38.71 (11.63)	37.83 (10.94)	0.685

Characteristics	rs10440833				rs6931514			
	AA	AT	TT	p value	AA	AG	GG	p value
Fasting blood glucose	10.03 (5.22)	9.88 (5.04)	10.02 (3.53)	0.964	9.89 (3.77)	9.56 (3.97)	10.57 (6.04)	0.248
Glycated haemoglobin	9.16 (3.10)	9.24 (2.09)	9.51 (2.49)	0.622	9.52 (2.54)	9.13 (2.03)	9.39 (2.98)	0.515
Total cholesterol	4.53 (1.36)	4.57 (1.26)	4.49 (1.37)	0.877	4.51 (1.43)	4.57 (1.28)	4.50 (1.31)	0.892
Triglyceride	2.48 (2.39)	2.54 (2.27)	2.44 (2.16)	0.944	2.41 (2.15)	2.39 (1.87)	2.70 (2.77)	0.543
LDL-C	2.45 (0.99)	2.70 (1.11)	2.63 (0.95)	0.149	2.61 (1.01)	2.74 (1.12)	2.47 (0.93)	0.072
HDL-C	1.18 (0.42)	1.18 (0.60)	1.25 (0.67)	0.575	1.21 (0.60)	1.22 (0.61)	1.17 (0.52)	0.722
Urea	5.84 (2.24)	6.03 (2.05)	6.98 (4.95)	0.015	7.22 (5.49)	5.88 (1.8)	6.12 (2.48)	0.005
Creatinine	71.13 (31.03)	67.19 (29.15)	70.02 (28.7)	0.503	71.47 (30.87)	64.89 (17.56)	72.97 (39.49)	0.037
Cystatin C	0.88 (0.77)	0.82 (0.22)	1.29 (3.99)	0.267	1.45 (4.63)	0.82 (0.21)	0.88 (0.68)	0.139
ALBP	18.45 (24.75)	31.32 (58.73)	29.18 (56.25)	0.275	19.62 (42.71)	26.52 (48.50)	26.52 (48.5)	0.354
GFR	124.7 (31.63)	123.45 (36.9)	119.40 (37.95)	0.612	115.75 (37.32)	126.08 (36.13)	122.1 (34.25)	0.174
CRP	1.23 (0.99)	1.5 (2.07)	1.30 (0.86)	0.436	1.28 (0.75)	1.55 (2.08)	1.18 (0.91)	0.196
INS	16.27 (10.63)	21.77 (24)	16.19 (12.70)	0.044	15.4 (13.01)	21.59 (23.52)	16.91 (12.01)	0.052
25QWD	23.71 (6.69)	24.24 (16.89)	26.25 (17.98)	0.622	27.22 (19.81)	24.59 (16.90)	23.02 (6.86)	0.353
UCRP	0.39 (0.70)	0.6 (1.51)	0.57 (1.22)	0.512	0.59 (1.34)	0.48 (1.03)	0.59 (1.53)	0.762
RBP	37.83 (10.94)	38.71 (11.63)	39.58 (10.61)	0.685	39.54 (10.52)	39.56 (11.72)	36.99 (10.6)	0.274

Bold values indicate that $p < 0.05$ indicates statistical significance.

SNP, single nucleotide polymorphism; T2D, type 2 diabetes; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; ALBP, adipocyte lipid-binding protein; GFR, glomerular filtration rate; CRP, C-reactive protein; INS, insulin; 25QWD, 25 hydroxy-vitamin D; UCRP, ubiquitin cross-reactive protein; RBP, retinol-binding protein.

Meta-analysis showed that rs7754840 polymorphism conferred to the increased the risk of T2D in the allele model (OR 1.29, 95% CI 1.14–1.45; Fig. 2). No correlation between 10946398 and T2D risk was found.

Discussion

In this study, we found significant associations between *CDKAL1* polymorphisms (rs4712523, rs4712524, rs10946398, rs7754840, rs35612982 and rs10440833) and T2D risk among the Han population from Northwest China. Specially, this study first provided evidence for the remarkable contribution of *CDKAL1* rs35612982 and rs10440833 to T2D susceptibility in the Chinese Han population. More interestingly, we found that genetic variants of *CDKAL1* could affect the risk of T2D not only by itself but also by associating with age, BMI and the status of smoking and drinking. Besides, rs35612982-CT and rs10440833-AT genotypes had a higher insulin level.

CDKAL1 gene is regarded as T2D susceptibility gene involved in glucose regulation and insulin secretion/action [41]. *CDKAL1* variants were related with defects in proinsulin conversion and insulin response upon glucose stimulation [42]. Several studies have provided

evidence for the significant contribution of *CDKAL1* polymorphisms to T2D risk [35, 43, 44]. Consistent with previous reports, *CDKAL1* variants (rs4712523, rs4712524, rs10946398, rs7754840) were associated with T2D susceptibility in the Chinese Han population. Moreover, our results first found that rs35612982 and rs10440833 polymorphisms conferred to the increased risk of T2D among the Chinese Han population, especially rs35612982 polymorphism that has not been reported. This result suggests that the *CDKAL1*rs35612982 variant might play an important role in the pathogenesis of T2D. Previously, Lu et al. [43] suggested that rs6931514 and rs7756992 were associated with increased T2D risk in Chinese populations. However, there was no association of rs6931514 and rs7756992 with the risk of T2D in our study. A global meta-analysis also revealed that no association for the SNP rs7756992 was found in the Asian population [45]. Such inconsistencies in these reports may result from different environmental conditions or insufficient sample size.

Genetic and environmental factors are interrelated and contribute to the development of T2D. Age, obesity, and unhealthy lifestyle identify those individuals at highest risk of T2D [46]. Interestingly, we found that rs4712524, rs35612982 and rs10440833 variants contributed the increased T2D risk at age ≤ 59 not > 59 years, suggesting that the risk association might be age dependent. Overweight and obesity (defined by BMI) is a known risk factor for T2D [47]. We found that BMI > 24 kg/m² was associated with increased risk of T2D. Stratified by BMI, we found that *CDKAL1* polymorphisms (rs4712523, rs4712524, rs10946398, rs7754840, rs35612982 and rs10440833) were associated with the increased susceptibility to T2D in the overweight/obese (BMI > 24 kg/m²) population. Smoking and drinking (especially heavy alcohol consumption) are known risk factors for diabetes. Smoking increases the risk of T2D by 30–40% for active smokers compared to non-smokers [48]. It has been found that cigarette smoking and alcohol consumption are related to glycaemic control and insulin resistance [49, 50]. However, drinking might have a reduced T2D risk, while smoking had no association with T2D in our study. In addition, our results revealed that *CDKAL1* polymorphisms increased the risk of T2D among those non-smokers and non-drinkers. The effect of smoking and drinking on the association between *CDKAL1* polymorphisms and the susceptibility of T2D are required to validate the results in larger populations and in laboratory-based functional experiments. However, the limitations of the present study should not be ignored. First, the subjects were enrolled from identical hospitals; therefore, they may not be representative of the general population. Second, the potential function of these SNPs in the aetiology of T2D was still unknown; thus, genetic and functional analyses were urgently required to reveal the precise role of these polymorphisms in T2D.

Conclusions

In conclusion, the present study identified the *CDKAL1* rs35612982 (C/T) polymorphism, as a novel polymorphism, associated with T2D in the Han population from Northwest China. Our study might help to enhance the understanding of *CDKAL1* gene mutations in the pathogenesis of T2D. Further studies are needed to evaluate the functional and epidemiological importance of the rs35612982 polymorphism in T2D.

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Statement of Ethics

This case-control study was carried out with the approval of the Ethics Committee of the First Affiliated Hospital of Xi'an Jiaotong University and in accordance with the guidelines of the Declaration of Helsinki. All participants were informed about the purpose of this study and signed a written informed consent.

Disclosure Statement

The authors declare that they have no conflict of interest.

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