BMJ Open Diabetes Research & Care

Association of the *CDKAL1* gene polymorphism with gestational diabetes mellitus in Chinese women

Chengjing Huang ,[1] Yan Guo,[2] Wei Li,[3] Bing Xiang,[1] Jing Zeng,[1] Feng Zhou,[1] Lu She,[1] Pei Zhang,[1] Shiwei Wang,[1] Bei Liu,[4] Qiong Dai,[5] Mei Yang[1]

To cite: Huang C, Guo Y, Li W, *et al*. Association of the *CDKAL1* gene polymorphism with gestational diabetes mellitus in Chinese women. *BMJ Open Diab Res Care* 2023;**11**:e003164. doi:10.1136/bmjdrc-2022-003164

► Additional supplemental material is published online only. To view, please visit the journal online (http://dx.doi.org/10.1136/bmjdrc-2022-003164).

Received 9 October 2022
Accepted 21 March 2023

For numbered affiliations see end of article.

Correspondence to
Professor Mei Yang;
644593079@qq.com

ABSTRACT

Introduction To identify the association of the cyclin-dependent kinase 5 regulatory subunit associated protein 1-like 1 (*CDKAL1*) gene polymorphism with gestational diabetes mellitus (GDM) in the Chinese population.

Research design and methods This case-control study enrolled 835 pregnant women with GDM and 870 pregnant women without diabetes who underwent antenatal examination during 24 to 28 gestational weeks at the Maternal and Child Health Hospital of Hubei Province from January 15, 2018 to March 31, 2019. Trained nurses collected their clinical information and blood samples. *CDKAL1* gene rs10440833, rs10946398, rs4712523, rs4712524, rs7754840, rs7756992 and rs9465871 loci were genotyped by Agena MassARRAY system. SPSS V.26.0 software and online SHesis were used to analyze the relationship between *CDKAL1* gene polymorphism and GDM susceptibility.

Results After being adjusted for maternal age, prepregnancy body mass index (BMI), parity and family history of type 2 diabetes mellitus (T2DM), *CDKAL1* gene rs10440833 (AA vs TT, OR=1.631, 95% CI 1.192 to 2.232), rs10946398 (CC vs AA, OR=1.400, 95% CI 1.028 to 1.905), rs4712523 (GG vs AA, OR=1.409, 95% CI 1.038 to 1.913), rs4712524 (GG vs AA, OR=1.418, 95% CI 1.043 to 1.929) and rs7754840 (CC vs GG, OR=1.407, 95% CI 1.036 to 1.911) polymorphisms were all associated with the increased risk of GDM. In addition, there was a powerful linkage disequilibrium (LD) among rs10946398, rs4712523, rs4712524 and rs7754840 (D′>0.900, r^2>0.900). And there were significant differences in haplotype CGGC (OR=1.207, 95% CI 1.050 to 1.387) and AAAG (OR=0.829, 95% CI 0.721 o 0.952, p=0.008) between the GDM group and the control group.

Conclusions rs10440833, rs10946398, rs4712523, rs4712524 and rs7754840 of *CDKAL1* gene are associated with GDM susceptibility in central Chinese population.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Being of high prevalence and an economic burden, gestational diabetes mellitus (GDM) has obviously become an important public health problem in China.

⇒ GDM has common risk factors and similar pathophysiological processes with type 2 diabetes mellitus (T2DM).

⇒ Many investigations have confirmed the association between multiple *CDKAL1* gene polymorphisms (rs7754840, rs4712524, rs10946398, rs7756992) and T2DM risk in different populations.

⇒ However, the association between *CDKAL1* gene polymorphisms and GDM risk is not completely clear.

WHAT THIS STUDY ADDS

⇒ The present study revealed that the polymorphisms of rs10440833, rs10946398, rs4712523, rs4712524 and rs7754840 of *CDKAL1* gene were significantly associated with the pathogenesis of GDM.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ Our study would provide a theoretical basis for early prediction and prevention of GDM in the Chinese population and provide some data for the genomic research of GDM in various races.

INTRODUCTION

Gestational diabetes mellitus (GDM) refers to the first diagnosis or confirmation of glucose intolerance during pregnancy,[1] which will significantly increase the risk of short-term and long-term metabolic diseases on pregnant women and their fetuses.[2] These diseases include gestational hypertension, spontaneous abortion, respiratory distress syndrome of newborns, and the development of complex diseases such as type 2 diabetes mellitus (T2DM) and cardiovascular disease.[3–6] The prevalence of GDM in China was 12.85% by 2019, and in the past 15 years, it was increasing year by year with the rapid development of economy and the change of people's lifestyle.[7] In 2015, the widespread popularity of GDM in China caused an economic burden of 5.59 billion yuan.[8] GDM has obviously become an important public health problem.

The pathogenetic mechanisms of GDM are not completely clear, but they are considered to be the results of the interaction between heredity and environment. GDM has common risk factors and similar pathophysiological

processes with T2DM,[9] suggesting that genetic factors of T2DM may also be involved in the occurrence and development of GDM. T2DM is a polygenic disorder characterized by impaired insulin secretion and insulin resistance. Since 2007, the results of genome-wide association studies (GWAS) at domestic and abroad have prompted that the cyclin-dependent kinase 5 regulatory subunit associated protein 1-like 1 (*CDKAL1*) is related to the genetic risk of T2DM.[10 11] *CDKAL1* gene is located on the short arm of human chromosome 6 and is highly expressed in human pancreatic islet and skeletal muscle. *CDKAL1* is an inhibitor of cyclin-dependent kinase 5 (CDK5),[12] and it affects the function of β cells by inhibiting the activity of CDK5 and tRNA-modifying enzymes.[13–15] Defects in *CDKAL1* gene can remarkably reduce insulin response to a glucose load.[13 16] Many investigations have confirmed the association between multiple *CDKAL1* gene polymorphisms (rs7754840, rs4712524, rs10946398, rs7756992) and T2DM risk in different populations.[17–19] It means that *CDKAL1* may also play a role in the occurrence and development of GDM by affecting blood glucose homeostasis.[20 21] However, the association between *CDKAL1* gene polymorphisms and GDM risk has been less studied, and the results are highly controversial.[18 22–26] Single nucleotide polymorphism (SNP) is the simplest form of DNA variation among individuals, which may change the encoded amino acids or influence gene expression and produce disease.[27] As far as we know, there are no studies involving *CKDAL1* rs4712523 and rs4712524 in the Chinese GDM population, and the results of studies between other SNPs of *CDKAL1* and GDM susceptibility have not been clarified. In the context of the situation, we explored the relationships between *CDKAL1* gene rs10440833, rs10946398, rs4712523, rs4712524, rs7754840, rs7756992 and rs9465871 polymorphisms and GDM in the Chinese population, so as to provide a theoretical basis for early prediction and prevention of GDM.

MATERIALS AND METHODS

Study population

The pregnant women who took antenatal examinations in Obstetrics and Gynecology Clinic of Maternal and Child Health Hospital of Hubei Province from January 15, 2018 to March 31, 2019 were recruited in the study. All subjects were enrolled at 24–28 gestational weeks after the 75 g-oral glucose tolerance test (OGTT). The diagnosis of GDM was based on the criteria of International Association of Diabetes and Pregnancy Study Groups (IADPSG): fasting plasma glucose (FPG) ≥5.1 mmol/L, or 1h-OGTT ≥10.0 mmol/L, or 2h-OGTT ≥8.5 mmol/L. Pregnant women who reached these thresholds were included in the GDM group. The randomly selected controls were category matched according to testing date and gestational weeks at the same outpatient clinic. Exclusion criteria are as follows: age <18 years; ethnic minorities; pre-gestational diabetes; multiple pregnancies; pregnancies complicated with endocrine diseases

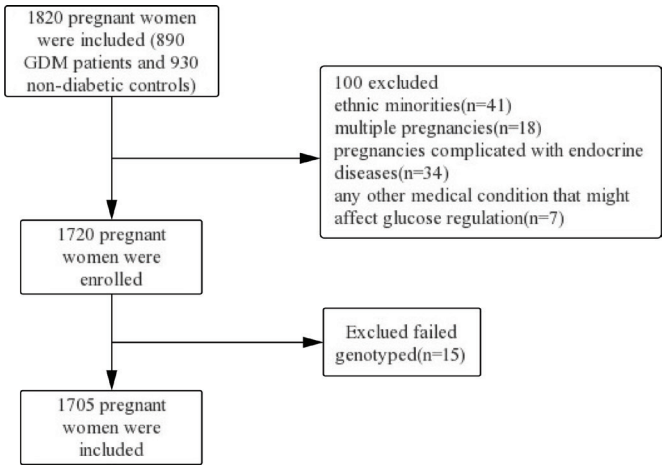

Figure 1 Selection of subjects for inclusion in the study. GDM, gestational diabetes mellitus.

such as hypertension and polycystic ovary syndrome; any other medical condition that might affect glucose regulation; unable or unwilling to participate in the study. At the genotyping stage, unsuccessful samples were also excluded. After exclusion, 1705 pregnant women (835 patients with GDM and 870 controls without diabetes) were included (figure 1). All the subjects were unrelated Han Chinese and lived in Wuhan of Hubei Province, a central area of China.

Data collection

A face-to-face questionnaire survey was conducted by uniformly trained nurses using the unified questionnaire to obtain the demographic characteristics, gravidity, parity, first degree family history of T2DM and other medical issues. Height and prepregnancy weight were obtained through medical records. The body mass index (BMI) was calculated as the weight in kilograms divided by the square of the height in meters.

The methods were carried out in accordance with the principles of the Declaration of Helsinki.

Laboratory testing

The candidate SNPs of *CDKAL1* gene were screened by SNPinfo, NCBI-dbSNP and HapMap databases. In 2002, in order to capture high-frequency variants of large effect and the association between rare mutations and diseases in the population, the HapMap project took SNPs with minor allele frequency (MAF) >0.05 as the primary research goal. By tracking the literature, combining with GWAS of T2DM and MAF >0.05 reported in the Chinese population,[26 28] we selected seven candidate SNPs (rs10440833, rs10946398, rs4712523, rs4712524, rs7754840, rs7756992 and rs9465871) that might be associated with GDM risk. During recruitment, 2 mL fasting peripheral venous blood was collected and placed in EDTA anticoagulant tube. After separation, it was packed in 1.5 mL EP tube and stored at −80°C until analysis. Genomic DNA was isolated from blood cells using the Relax Gene Blood DNA System DP348 (Tiangen, China).

Genotyping of candidate SNPs was conducted on the Sequenom MassARRAY platform (Sequenom, San Diego, California, USA). In order to control quality, 5% of the samples was randomly selected and repeated genotyped.

Statistical analysis

The quantitative data were expressed by mean±SD ($\bar{x}$±SD), and the categorical data were expressed by frequency (n) and percentage (%). The differences in demographic characteristics, allele frequency and genotypic distribution of candidate SNPs between GDM and control groups were compared by independent sample t-test or χ^2 test. Hardy-Weinberg equilibrium (HWE) was tested using Pearson's χ^2 test with a threshold of p>0.05 in controls. The general association of genotypes with GDM was assessed with multivariate logistic regression analysis under allele, recessive, codominant, dominant and overdominant models. False discovery rate (FDR) correction was applied to revise the adjusted p values in the case of multiple comparisons. Analysis of covariance was used to explore the relationship between SNPs and blood glucose levels. Dunnett-t method was conducted to compare the average of blood glucose levels in different genotypes. Linkage disequilibrium (LD) is the statistical associations between mutations, which plays a central role in the genetic basis of diseases and other complex traits.[29] SHesis online software was used to analyze the LD among *CDKAL1* gene SNPs and construct haplotypes (http://analysis.bio-x.cn/myAnalysis.php). The data of this study were analyzed by SPSS V.26.0 software. P value <0.05 was considered statistically significant.

RESULTS

General characteristics of the study subjects

The demographic characteristics of the subjects were presented in table 1. The age, prepregnancy BMI, FPG, 1h-OGTT and 2h-OGTT of patients with GDM were higher than those of the control group (p<0.05). The gravidity and parity between GDM and control group were of significant differences (p<0.05). The proportion of pregnant women with family history of T2DM in the case group was higher than that in the control group (29.82% vs 11.84%), and the difference was statistically significant (χ^2=84.087, p<0.001).

Association of *CDKAL1* gene SNPs with GDM

The distributions of seven candidate SNPs in the control group were all in HWE (p>0.05). The associations of rs10440833, rs10946398, rs4712523, rs4712524, rs7754840, rs7756992 and rs9465871 polymorphisms with GDM were shown in table 2. After adjusted for age, prepregnancy BMI, parity and family history of T2DM, the genotype distributions of *CDKAL1* gene rs10440833 (AA vs TT, OR=1.631, 95% CI 1.192 to 2.232), rs10946398 (CC vs AA, OR=1.400, 95% CI 1.028 to 1.905), rs4712523 (GG vs AA, OR=1.409, 95% CI 1.038 to 1.913), rs4712524 (GG vs AA, OR=1.418, 95% CI 1.043 to 1.929) and rs7754840 (CC vs GG, OR=1.407, 95% CI 1.036 to 1.911) were all associated with the increased risk of GDM. In addition, there were no significant differences in genotype distributions of rs7756992 and rs9465871 between the two groups (p>0.05). The results were stable after being adjusted for FDR correction.

Table 1 Demographic characteristics of the subjects

	GDM（n=835）	Non-GDM（n=870）	χ^2/t	P value
Age (years)	30.97±4.56	28.84±4.20	10.45	**<0.001**
Prepregnancy BMI (kg/m^2)	22.16±3.35	20.67±2.37	10.644	**<0.001**
Gravidity				
1	294 (35.21)	342 (39.31)	7.917	**0.048**
2	257 (30.78)	274 (31.49)		
3	153 (18.32)	155 (17.82)		
≥4	131 (15.69)	99 (11.38)		
Parity				
0	493 (59.04)	532 (61.15)	7.249	**0.027**
1	303 (36.29)	318 (36.55)		
≥2	39 (4.67)	20 (2.30)		
FPG (mmol/L)	5.05±0.88	4.34±0.31	17.315	**<0.001**
1h-OGTT (mmol/L)	10.42±1.69	7.38±1.34	34.064	**<0.001**
2h-OGTT (mmol/L)	9.14±1.73	6.50±0.98	30.969	**<0.001**
Family history of T2DM				
No	586 (70.18)	767 (88.16)	84.087	**<0.001**
Yes	249 (29.82)	103 (11.84)		

BMI, body mass index; FPG, fasting plasma glucose; GDM, gestational diabetes mellitus; OGTT, oral glucose tolerance test; T2DM, type 2 diabetes mellitus.

Table 2 The association of the CDKAL1 gene polymorphism with GDM

SNPs	Genotypes/ allele	GDM (%)	Non-GDM (%)	Crud OR (95% CI)	Crud p values	Adjusted OR (95 % CI)	Adjusted p values
rs10440833	TT	280 (34.53)	336 (39.53)	1 (ref.)		1 (ref.)	
	AT	380 (46.86)	403 (47.41)	1.132 (0.916 to 1.398)	0.253	1.098 (0.874 to 1.379)	0.42
	AA	151 (18.62)	111 (13.06)	1.632 (1.219 to 2.186)	0.001	1.631 (1.192 to 2.232)	**0.002**
rs10946398	AA	278 (33.57)	329 (38.17)	1 (ref.)		1 (ref.)	
	CA	400 (48.31)	411 (47.68)	1.152 (0.933 to 1.422)	0.189	1.108 (0.884 to 1.390)	0.372
	CC	150 (18.12)	122 (14.15)	1.382 (1.092 to 1.940)	0.011	1.400 (1.028 to 1.905)	**0.033**
rs4712523	AA	270 (32.61)	323 (37.56)	1 (ref.)		1 (ref.)	
	GA	402 (48.55)	409 (47.56)	1.176 (0.951 to 1.454)	0.135	1.124 (0.895 to 1.411)	0.313
	GG	156 (18.84)	128 (14.88)	1.458 (1.097 to 1.937)	0.009	1.409 (1.038 to 1.913)	**0.028**
rs4712524	AA	275 (33.21)	325 (37.75)	1 (ref.)		1 (ref.)	
	GA	399 (48.19)	413 (47.97)	1.142 (0.924 to 1.411)	0.219	1.090 (0.869 to 1.367)	0.456
	GG	154 (18.60)	123 (14.29)	1.404 (1.111 to 1.970)	0.007	1.418 (1.043 to 1.929)	**0.026**
rs7754840	GG	275 (33.17)	326 (37.69)	1 (ref.)		1 (ref.)	
	CG	399 (48.13)	415 (47.98)	1.140 (0.923 to 1.408)	0.225	1.090 (0.869 to 1.367)	0.456
	CC	155 (18.70)	124 (14.34)	1.482 (1.114 to 1.971)	0.007	1.407 (1.036 to 1.911)	**0.029**
rs7756992	AA	199 (24.27)	231 (26.95)	1 (ref.)		1 (ref.)	
	GA	410 (50.00)	438 (51.11)	1.087 (0.861 to 1.371)	0.484	1.078 (0.840 to 1.384)	0.554
	GG	211 (25.73)	188 (21.94)	1.303 (0.991 to 1.712)	0.058	1.272 (0.949 to 1.704)	0.108
rs9465871	TT	189 (22.77)	224 (26.02)	1 (ref.)		1 (ref.)	
	CT	422 (50.84)	443 (51.45)	1.129 (0.893 to 1.428)	0.312	1.066 (0.829 to 1.372)	0.618
	CC	219 (26.39)	194 (22.53)	1.338 (1.018 to 1.759)	0.037	1.276 (0.952 to 1.711)	0.103

Adjusted OR is adjusted for age, prepregnancy BMI, parity, family history of T2DM.
BMI, body mass index; GDM, gestational diabetes mellitus; ref, reference genotype; SNPs, single nucleotide polymorphisms; T2DM, type 2 diabetes mellitus.

The results of allele, recessive, dominant and overdominant models were presented in the online supplemental table 1. The rs10440833/A, rs10946398/C, rs4712523/G, rs4712 524/G and rs7754840/C led to significantly higher risks for GDM (p<0.05). For recessive model, the distributions of *CDKAL1* gene rs10440833 (AA vs AT+TT, OR=1.523, 95% CI 1.167 to 1.989), rs4712523 (GG vs GA+AA, OR=1.328, 95% CI 1.028 to 1.715), rs4712524 (GG vs GA+AA, OR=1.371, 95% CI 1.058 to 1.776) and rs7754840 (CC vs CG+GG, OR=1.374, 95% CI 1.062 to 1.779) were all associated with the increased risk of GDM after adjusted for age, prepregnancy BMI, parity and family history of T2DM.

Relationships between candidate SNPs polymorphisms and blood glucose levels

An analysis of blood glucose levels (FPG, 1h-OGTT and 2h-OGTT) of pregnant women with different genotypes was performed (table 3). The results showed that 1h-OGTT and 2h-OGTT levels were significant different among rs10440833 genotypes (p<0.05). 2h-OGTT levels in rs4712523 GG genotype were significantly higher than those of GA and AA genotype (p<0.05). For rs7754840, 2h-OGTT levels of CT and TT genotype carriers were significantly less than CC genotype carriers (p<0.05).

LD analysis and haplotype construction among SNPs of *CDKAL1* gene

The LD analysis among candidate SNPs of *CDKAL1* gene was shown in figure 2. The results showed that there was a strong LD among rs10946398, rs4712523, rs4712524 and rs7754840 (D′>0.900, r^2>0.900). Although there was a non-random association between rs7756992 and rs9465871 alleles (D′=0.982, r^2=0.929), no significant correlation was founded between them and the risk of GDM. Haplotype construction was only carried out between rs10946398, rs4712523, rs4712524 and rs7754840, and the results were shown in table 4. There were four haplotypes: AAAG, CGGC, AGAG and CAGC. Among them, the distributions of haplotype CGGC (OR=1.207, 95% CI 1.050 to 1.387, p=0.008) and AAAG (OR=0.829, 95% CI 0.721 to 0.952, p=0.008) were statistically different between the two groups.

DISCUSSION

Our study analyzed the relationships between seven *CDKAL1* gene SNPs (rs10440833, rs10946398, rs4712523, rs4712524, rs7754840, rs7756992, rs9465871) with GDM risks in Wuhan population of China. The results showed that homozygous polymorphisms in rs10440833,

Table 3 Comparison of blood glucose levels among different genotypes of candidate SNPs

SNPs	OGTT Blood sugar levels	Genotypes AA	AB	BB	F values	P values
rs10440833	FPG	4.87±0.73	4.79±0.80	4.71±0.72	2.879	0.057
	1h-OGTT	9.63±2.02*	9.28±2.16	9.07±2.11	3.988	**0.019**
	2h-OGTT	8.49±1.89*	8.16±1.97	7.91±1.86	5.611	**0.004**
rs10946398	FPG	4.83±0.73	4.81±0.80	4.73±0.83	0.72	0.487
	1h-OGTT	9.54±2.02	9.28±2.18	9.13±2.17	1.407	0.245
	2h-OGTT	8.45±1.91*	8.16±1.95	7.97±1.99	3.012	0.05
rs4712523	FPG	4.83±0.73	4.81±0.80	4.73±0.84	0.704	0.495
	1h-OGTT	9.54±2.03	9.30±2.17	9.11±2.18	1.926	0.146
	2h-OGTT	8.47±1.90*	8.17±1.95	7.96±2.00	3.726	**0.024**
rs4712524	FPG	4.83±0.73	4.81±0.80	4.73±0.83	0.81	0.445
	1h-OGTT	9.54±2.01	9.29±2.17	9.13±2.16	1.592	0.204
	2h-OGTT	8.45±1.90*	8.16±1.95	7.98±2.00	3.005	0.05
rs7754840	FPG	4.84±0.73	4.81±0.80	4.73±0.83	0.671	0.512
	1h-OGTT	9.58±2.00	9.26±2.18	9.13±2.17	1.827	0.161
	2h-OGTT	8.46±1.87*	8.14±1.96	7.97±2.00	3.117	**0.045**

A: risk allele; B: major allele.
*The blood glucose level of AA or AB genotype was compared with that of BB genotype, p<0.05, and the difference was statistically significant.
OGTT, oral glucose tolerance test; SNP, single nucleotide polymorphism.

rs10946398, rs4712523, rs4712524 and rs7754840 increased the risk of GDM. In addition, we analyzed the LD among candidate SNPs and constructed haplotypes. The results showed that there was a strong LD among rs10946398, rs4712523, rs4712524 and rs7754840, and the distribution of haplotype CGGC and AAAG was significantly different between GDM and control groups.

Consistent with another study in the Chinese population,[22] we confirmed the robust association of *CDKAL1* rs10440833/A variant with GDM risk. In a 2007 study, the rs10440833 variant increased the risk of T2DM through reduced insulin secretion in obese individuals of European ancestry and individuals from Hong Kong of Han Chinese ancestry.[16] Moreover, a significant relationship was observed among rs10440833 and T2DM risk under the recessive model in Indian.[30] For rs10946398, our study found that CC genotype was significantly associated with the increased risk of GDM, similar to the results of Zhang *et al* from another Chinese populations.[23] Zhang *et al* found that the insulin secretion index and insulin sensitivity index of CC genotype were significantly lower than those of AA genotype as well.[23] The evidence from Cardiometabolic Risk in Chinese (CRC) study showed that rs10946398 polymorphism was associated with the impaired insulin secretion.[14] However, Tarnowski *et al* did not found significant association between rs10946398 polymorphism and GDM risk in European populations.[24] Therefore, the findings of rs10946398 should be dealt with cautiously and needed to be verified in large-scale genomic studies.

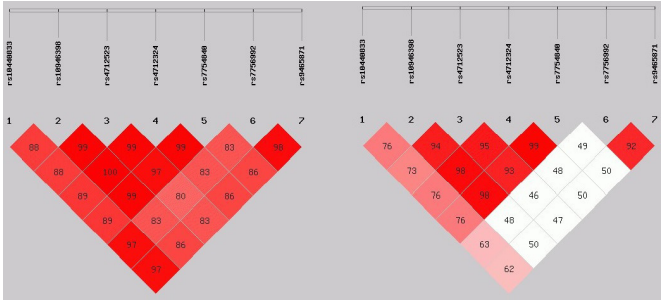

Figure 2 Inter-single nucleotide polymorphism linkage disequilibrium analysis of *CDKAL1* gene (D' test on the left, R^2 test on the right).

The study on the rs4712523 polymorphism and T2DM susceptibility in Han population in Inner Mongolia of China showed that the risk of T2DM in G allele carriers was 1.654 times higher than that in A allele.[31] The same association also confirmed in Finns and Japanese population.[10 32] For rs4712524, the G allele, GG and GG+AG genotypes have been confirmed with an increased risk of T2DM susceptibility in east Asian, European and Russian populations in previous observations.[33 34] In addition, Li [35] and Li *et al*[17] also concluded that the patients with G polymorphism have significantly higher FPG levels in the Chinese population. To our knowledge, our study was the first study examining the relationship between rs471253 and rs4712524 with GDM risk. The results showed that GG carriers of the two SNPs increased the risk of GDM

Table 4 Haplotype analysis for rs10946398, rs4712523, rs4712524, rs7754840 of *CDKAL1* gene

Haplotype	GDM (%)	Non-GDM (%)	χ^2	P values	OR (95% CI)
CGGC	694.97 (42.23)	643.97 (37.72)	7.029	**0.008**	1.207 (1.05 to 1.387)
AAAG	930.97 (56.51)	1040.97 (60.91)	7.029	**0.008**	0.829 (0.721 to 0.952)
AGAG	16.03 (0.99)	15.03 (0.90)	0.094	0.759	1.050 (0.210 to 5.191)
CAGC	3.03 (0.19)	3.03 (0.18)	0.125	0.724	1.121 (0.552 to 2.273)

compared with carriers of AA genotype. It was necessary to carry out larger sample verification in more races.

The correlation between *CDKAL1* gene rs7754840 and GDM risk has been explored in many studies, but the results varied considerably among different populations. Consistent with our conclusions, studies in South Korea, Malaysia, southern India and Bangladesh[18 20 36 37] showed a strong correlation between rs7754840 and the risk of GDM. Furthermore, a meta-analysis of Asian populations showed that the C allele of rs7754840 was significantly associated with the increased risk of GDM.[38] But the results of Wu *et al* and Wang *et al* in the Chinese population did not conclude the consistent correlations.[21 22] The quantity of the subjects in Wu *et al* study was 333 (including 153 patients with GDM and 180 controls without diabetes), which may affect the reliability of the results.[22] Different diagnostic criteria of GDM would have a great impact on the selection of subjects and even change the results of the correlation between gene polymorphism and GDM. GDM diagnosis of Wang *et al* was based on the criteria of American Diabetes Association: fasting 5.3 mmol/L, 1h-OGTT 10.0 mmol/L, 2h-OGTT 8.6 mmol/L and 3h-OGTT 7.8 mmol/L. A diagnosis of GDM was made if two or more of the glucose values met or exceeded the threshold value. Normal glucose tolerance was diagnosed when all plasma glucose values were below the threshold values.[21] Our study also found that C allele of rs7754840 locus was significantly associated with the higher blood glucose level, which was consistent with the results of previous studies.[20 21]

There were no associations between rs7756992 and rs9465871 polymorphisms and GDM risk in our study, similar to the results of a study in the Chinese population.[23] In 2010, Tan *et al* found that the rs7756992 was not associated with the risk of T2DM in Malays and Asian-Indians in Singapore,[39] which was consistent with the results in other population.[40] The lack of an association between *CDKAL1* rs9465871 and the incidence of T2DM in Korean was in line with a prospective study in the USA.[41 42] However, another meta-analysis indicated a consistently strong relationship between rs7756992 with GDM risk in Asian and Caucasian populations.[26] Amin *et al*[18] also confirmed that the AG and GG genotypes of *CDKAL1* rs7756992 increased the odds of GDM by 2.5-fold and 3.7-fold, respectively, in Bangladeshi population. This inconsistency may be due to differences in race, sample size and diagnostic criteria for GDM.

Furthermore, we explored the LD among the candidate SNPs of *CDKAL1* gene. We found that there was strong LD among rs10946398, rs4712523, rs4712524 and rs7754840, which might have a synergistic effect on the pathogenesis of GDM. The CGGC and AAAG haplotypes were significantly correlated with the risk of GDM. GDM has been proved to be related to a variety of genes,[34] the effect of haplotypes between SNPs on GDM susceptibility and its mechanism need to be further studied.

Although a relatively large sample size and the LD analysis were used in our study to ensure the credibility, there were also some limitations in this study. First, the subjects of this study were all from one hospital. Although conformed to HWE and the sample size was sufficient, the admission rate bias might exist. It was necessary to carry out more comprehensive, larger-scale and higher quality researches among the Chinese population. Second, this study only detected the blood glucose level of pregnant women, lacking metabolic indicators (such as blood lipids, adiponectin, insulin resistance index, FINS glycosylated hemoglobin level and so on) and lifestyle information (such as diet, physical exercise, sleep rhythm and so on) which were important factors affecting the development of GDM. Last but not least, GDM is considered to be the result of the joint action of multiple variation factors. These factors not only play a role alone, but also interact with other factors, such as gene-gene interaction or gene-environment interaction. This also deserved further study from different aspects and different populations. Our study did not clarify the potential impact of *CDKAL1* genetic variants in the pathogenesis of GDM, which needed to be further explored.

CONCLUSION

In summary, the present study revealed that the polymorphisms of rs10440833, rs10946398, rs4712523, rs4712524 and rs7754840 of *CDKAL1* gene were significantly associated with the pathogenesis of GDM.

Author affiliations
[1]Research Center for Health Promotion in Women, Youth and Children, Hubei Province Key Laboratory of Occupational Hazard Identification and Control, School of Public Health, Wuhan University of Science and Technology, Wuhan, Hubei, china
[2]School health center, Wuhan Centers for Disease Prevention and Control, Wuhan, Hubei, China
[3]Department of Pharmacy, Maternal and Child Health Hospital of Hubei Province, Wuhan, Hubei, China
[4]Department of Popular Science, Jinan Health Publicity and Education Center, Jinan, China

[5]Department of Popular Science, Maternal and Child Health Hospital of Hubei Province, Wuhan, Hubei, China

Contributors CH, WL and YG contributed equally to this paper. QD and MY are both the corresponding authors.MY was the guarantor for overall content. CH, WL and YG contributed to study design, analysis and interpret data, and drafted the manuscript. BX, JZ, FZ, LS, PZ, SW and BL performed data collection, analysis and interpretation. MY and QD participated in the design and coordination of the study and revised the manuscript. All authors read and approved the final manuscript.

Funding This study was funded by The National Natural Science Fund of China (grant number: 81703239), Chinese Center for Disease Control and Prevention (grant number: 2018FYH014), Health Commission of Hubei Province (grant number: WJ2018H0134, WJ2018H0145), and Scientific Research Program of Hubei Education Department (grant number: B2020006).

Competing interests None declared.

Patient consent for publication Not applicable.

Ethics approval The study was approved by the institutional review board of Wuhan University of Science and Technology (2017002) and informed consent was issued and signed by all subjects. Participants gave informed consent to participate in the study before taking part.

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement All data relevant to the study are included in the article or uploaded as supplemental information.

ORCID iD

Chengjing Huang http://orcid.org/0000-0003-1794-4975

REFERENCES

1 Queensland Clinical Guidelines. *Gestational diabetes mellitus (GDM) [M]. (guideline no.MN21.33-V5-R26)*. Australia: Queensland Health, 2021: 1–46.
2 Jun J, Yang HX, Tang X. Gestational diabetes: an opportunity to improve maternal and infant health. *Chinese Journal of Perinatal Medicine* 2020;23:717–8.
3 Beucher G, Viaris de Lesegno B, Dreyfus M. Maternal outcome of gestational diabetes mellitus. *Diabetes Metab* 2010;36:522–37.
4 Bellamy L, Casas J-P, Hingorani AD, *et al*. Type 2 diabetes mellitus after gestational diabetes: a systematic review and meta-analysis. *Lancet* 2009;373:1773–9.
5 Johns K, Olynik C, Mase R, *et al*. Gestational diabetes mellitus outcome in 394 patients. *J Obstet Gynaecol Can* 2006;28:122–7.
6 Reece EA, Leguizamón G, Wiznitzer A. Gestational diabetes: the need for a common ground. *Lancet* 2009;373:1789–97.
7 Wang SB, Zhou QM, Sheng WW, *et al*. Investigation and analysis of risk factors and pregnancy outcome of gestational diabetes mellitus in china. *Chinese Medical Journals* 2019;54:1014–9.
8 Xu T, Dainelli L, Yu K, *et al*. The short-term health and economic burden of gestational diabetes mellitus in china: a modelling study. *BMJ Open* 2017;7:e018893.
9 Shaat N, Groop L. Genetics of gestational diabetes mellitus. *Curr Med Chem* 2007;14:569–83.
10 Scott LJ, Mohlke KL, Bonnycastle LL, *et al*. A genome-wide association study of type 2 diabetes in finns detects multiple susceptibility variants. *Science* 2007;316:1341–5.
11 Diabetes Genetics Initiative of Broad Institute of Harvard and MIT, Lund University, and Novartis Institutes of BioMedical Research, Saxena R, Voight BF, *et al*. Genome-Wide association analysis identifies loci for type 2 diabetes and triglyceride levels. *Science* 2007;316:1331–6.
12 Liu K-C, Leuckx G, Sakano D, *et al*. Inhibition of Cdk5 promotes β-cell differentiation from ductal progenitors. *Diabetes* 2018;67:58–70.
13 Pascoe L, Tura A, Patel SK, *et al*. Common variants of the novel type 2 diabetes genes CDKAL1 and HHEX/IDE are associated with decreased pancreatic beta-cell function. *Diabetes* 2007;56:3101–4.
14 Liang J, Pei Y, Liu X, *et al*. The CDKAL1 gene is associated with impaired insulin secretion and glucose-related traits: the cardiometabolic risk in Chinese (CRC) study. *Clin Endocrinol (Oxf)* 2015;83:651–5.
15 Stancáková A, Pihlajamäki J, Kuusisto J, *et al*. Single-Nucleotide polymorphism rs7754840 of CDKAL1 is associated with impaired insulin secretion in nondiabetic offspring of type 2 diabetic subjects and in a large sample of men with normal glucose tolerance. *J Clin Endocrinol Metab* 2008;93:1924–30.
16 Steinthorsdottir V, Thorleifsson G, Reynisdottir I, *et al*. A variant in CDKAL1 influences insulin response and risk of type 2 diabetes. *Nat Genet* 2007;39:770–5.
17 Li C, Shen K, Yang M, *et al*. Association between single nucleotide polymorphisms in CDKAL1 and Hhex and type 2 diabetes in Chinese population. *DMSO* 2021;Volume 13:5113–23.
18 Amin USM, Parvez N, Rahman TA, *et al*. CDKAL1 gene rs7756992 A/G and rs7754840 G/C polymorphisms are associated with gestational diabetes mellitus in a sample of bangladeshi population: implication for future T2DM prophylaxis. *Diabetol Metab Syndr* 2022;14:18.
19 Xu M, Bi Y, Xu Y, *et al*. Combined effects of 19 common variations on type 2 diabetes in Chinese: results from two community-based studies. *PLoS One* 2010;5:e14022.
20 Kanthimathi S, Chidambaram M, Liju S, *et al*. Identification of genetic variants of gestational diabetes in South Indians. *Diabetes Technol Ther* 2015;17:462–7.
21 Wang Y, Nie M, Li W, *et al*. Association of six single nucleotide polymorphisms with gestational diabetes mellitus in a Chinese population. *PLoS One* 2011;6:e26953.
22 Wu YL, Li SP, Zhang Z, *et al*. Association between gene polymorphism of cdkall and gestational diabetes mellitus. *Chin J Diabetes* 2015;23:501–4.
23 Zhang HL. *Correlation between SLC30A8 gene and CDKAL1 gene and gestat-ional diabetes mellitus*. Peking Union Medical College, 2009.
24 Tarnowski M, Malinowski D, Pawlak K, *et al*. GCK, GCKR, FADS1, DGKB/TMEM195 and CDKAL1 gene polymorphisms in women with gestational diabetes. *Can J Diabetes* 2017;41:372–9.
25 Tang Z, Li W, Zhou J, *et al*. Meta-analysis on the association between CDKAL1 gene rs7754840 and rs7756992 polymorphisms and gestational diabetes mellitus. *Modern Preventive Medicine* 2020;47:1124–9.
26 Guo F, Long W, Zhou W, *et al*. Fto, GCKR, CDKAL1 and CDKN2A/B gene polymorphisms and the risk of gestational diabetes mellitus: a meta-analysis. *Arch Gynecol Obstet* 2018;298:705–15.
27 Shastry BS. Snps: impact on gene function and phenotype. *Methods Mol Biol* 2009;578:3–22.
28 Wang K, Chen Q, Feng Y, *et al*. Single nucleotide polymorphisms in *cdkal1* gene are associated with risk of gestational diabetes mellitus in Chinese population. *J Diabetes Res* 2019;2019:3618103.
29 Good BH. Linkage disequilibrium between rare mutations. *Genetics* 2022;220:iyac004.
30 Verma AK, Goyal Y, Bhatt D, *et al*. Association between CDKAL1, Hhex, CDKN2A/2B and IGF2BP2 gene polymorphisms and susceptibility to type 2 diabetes in uttarakhand, India. *Diabetes Metab Syndr Obes* 2021;14:23–36.
31 Li XJ, Su Y, Yan ZL, *et al*. CDKAL1 rs4712523 polymorphism is associated with type 2 diabetes in han population in inner Mongolia. *Basic & Clinical Medicine* 2013;33:346–9.
32 Takeuchi F, Serizawa M, Yamamoto K, *et al*. Confirmation of multiple risk loci and genetic impacts by a genome-wide association study of type 2 diabetes in the Japanese population. *Diabetes* 2009;58:1690–9.
33 Unoki H, Takahashi A, Kawaguchi T, *et al*. Snps in KCNQ1 are associated with susceptibility to type 2 diabetes in East Asian and European populations. *Nat Genet* 2008;40:1098–102.
34 Azarova L. Polymorphic variants of rs4712524 and rs6931514 in the CDKAL1 gene: association with the risk of developmentof type 2 diabetes mellitus in Russian population. *Естественные и Технические Науки (in Russian)* 2020.

35 Li YR. *Characteristics of corporal heart disease complicated with type 2 diabetes comorbidity in the elderly and association of gene polymorphisms*. Peking Union Medical College, 2009.
36 Kwak SH, Kim S-H, Cho YM, *et al*. A genome-wide association study of gestational diabetes mellitus in Korean women. *Diabetes* 2012;61:531–41.
37 Aris NKM, Ismai NAM, Mahdy ZA, *et al*. An analysis of targeted single nucleotide polymorphisms for the risk prediction of gestational diabetes mellitus in a cohort of Malaysian patients. *Asia-Pacific J Mol Med* 2011;1:1–8.
38 Yu X-Y, Song L-P, Wei S-D, *et al*. CDK5 regulatory subunit-associated protein 1-like 1 gene polymorphisms and gestational diabetes mellitus risk: a trial sequential meta-analysis of 13,306 subjects. *Front Endocrinol (Lausanne)* 2021;12:722674.
39 Tan JT, Ng DPK, Nurbaya S, *et al*. Polymorphisms identified through genome-wide association studies and their associations with type 2 diabetes in Chinese, Malays, and Asian-Indians in Singapore. *J Clin Endocrinol Metab* 2010;95:390–7.
40 El-Lebedy D, Ashmawy I. Common variants in TCF7L2 and CDKAL1 genes and risk of type 2 diabetes mellitus in Egyptians. *J Genet Eng Biotechnol* 2016;14:247–51.
41 Kim J, Kim MK, Jung S, *et al*. Interaction of iron status with single nucleotide polymorphisms on incidence of type 2 diabetes. *PLoS ONE* 2017;12:e0175681.
42 Moore AF, Jablonski KA, McAteer JB, *et al*. Extension of type 2 diabetes genome-wide association scan results in the diabetes prevention program. *Diabetes* 2008;57:2503–10.