·Clinical Research·

The association analysis polymorphism of *CDKAL1* and diabetic retinopathy in Chinese Han population

Nai-Jia Liu [1], Qian Xiong [2], Hui-Hui Wu [1], Yan-Liang Li [1], Zhen Yang [3], Xiao-Ming Tao [4], Yan-Ping Du [4], Bin Lu [1], Ren-Ming Hu [1], Xuan-Chun Wang [1], Jie Wen [1]

[1]Department of Endocrinology and Metabolism, Huashan Hospital Affiliated to Fudan University, Shanghai 200040, China
[2]Department of Endocrinology and Metabolism, Jing'an District Center Hospital of Shanghai, Shanghai 200040, China
[3]Department of Endocrinology and Metabolism, Xin Hua Hospital Affiliated to Shanghai Jiao Tong University School of Medicine, Shanghai 200020, China
[4]Department of Endocrinology and Metabolism, Hua Dong Hospital Affiliated to Fudan University, Shanghai 200040, China
Co-first authors: Nai-Jia Liu and Qian Xiong
Correspondence to: Xuan-Chun Wang and Jie Wen. Department of Endocrinology and Metabolism, Huashan Hospital, Fudan University, No.12 Wulumuqi Mid Road, Building 0#, Jing'an District, Shanghai 200040, China. wangxch@fudan.edu.cn; wenjie065@126.com
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Abstract

• AIM: To identify the contribution of *CDKAL1* to the development of diabetic retinopathy (DR) in Chinese population.

• METHODS: A case-control study was performed to investigate the genetic association between DR and polymorphic variants of *CDKAL1* in Chinese Han population with type 2 diabetes mellitus (T2DM). A well-defined population with T2DM, consisting of 475 controls and 105 DR patients, was recruited. All subjects were genotyped for the genetic variant (rs10946398) of *CDKAL1*. Genotyping was performed by iPLEX technology. The association between rs10946398 and T2DM was assessed by univariate and multivariate logistic regression (MLR) analysis.

• RESULTS: There were significant differences in C allele frequencies of rs10946398 (*CDKAL1*) between control and DR groups (45.06% versus 55.00%, P<0.05). The rs10946398 of *CDKAL1* was found to be associated with the increased risk of DR among patients with diabetes.

• CONCLUSION: Our findings suggest that rs10946398 of *CDKAL1* is independently associated with DR in a Chinese Han population.

• KEYWORDS: *CDKAL1*; polymorphism; association analysis; diabetic retinopathy; Chinese Han population
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INTRODUCTION

Type 2 diabetes mellitus (T2DM), caused by a complex interaction between environmental and genetic factors, is a polygenic disorder characterized by defects in insulin secretion and insulin resistance. T2DM is associated with hyperglycaemia, oxidant stress, metabolic inflammation and significantly increased risk for macro-vascular complications and micro-vascular complications [1].

The diabetic retinopathy (DR), the second leading cause of vision loss due to the degeneration of the retina is also one of the most frequent micro-vascular complications [2]. Although the multifactorial etiologies of DR were poorly understood, there were several lines of evidence, such as ethnic differences [3-4] and familial clustering in identical twins with T2DM [5], implicating that genetic factors play some roles in the pathogenesis of DR. Therefore, elucidation of the genetic susceptibility factors for DR was important to gain insight into the pathogenesis of DR, and might define the genetic risk factors for this condition.

A number of studies have attempted to identify driving genes and their variants that are associated with DR across different populations, and these include the cyclin-dependent kinase 5 (CDK5) regulatory subunit-associated protein 1-like 1 (*CDKAL1*) gene [6]. *CDKAL1*, mapped to chromosome 6p22.3, encodes a protein that inhibits the activation of CDK5 through its homology to CDK5RAP1 [7], a well-characterized negative regulator of CDK5 [8] that functions through the inhibition of the CDK5 activator p35 [7,9]. Genetic defects in *CDKAL1* gene that was highly expressed in human pancreatic islet and skeletal muscle, remarkably reduced insulin response to a glucose load [10-11]. A series of genome wide association studies (GWASs) showed an association of the single nucleotide polymorphisms (SNPs) in *CDKAL1* gene with T2DM [12-15], including rs4712523,

rs10946398, rs7754840 and rs7756992 [16]. The association between the rs10946398 with cardiovascular risk has been reported in Chinese population[17].

Previous studies have been conducted to evaluate the association of rs10946398 in *CDKAL1* gene with T2DM. However, there is little known about the correlation between rs10946398 and diabetic complications. In the present study, we explored the potential relationship between rs10946398 and the development of DR among the Chinese Han population.

SUBJECTS AND METHODS

Subjects The studied population involved 580 unrelated Chinese Han patients with T2DM (62.31% females; average age: 64.73±10.85y when recruited). Participants, including 105 DR patients and 475 diabetic patients without retinopathy (DWR) patients, were recruited from rural and urban communities in Shanghai. T2DM patients registered in the analysis were recruited from the Endocrinology and Metabolism outpatient clinics at Huashan Hospital of Fudan University in Shanghai. Written consent was obtained from all patients before the study. This study was approved by the Ethics Committee of Huashan Hospital Affiliated to Fudan University, Shanghai, China.

Participants with the following conditions were excluded: known other types of diabetes; diabetic ketoacidosis or ketonuria; nutritional derangements; anemia; malignancy; thyroid dysfunction; pregnancy; breast-feeding; mental illness.

Measurement All participants were interviewed for the documentation of medical histories, medications and drinking history. A complete clinical baseline characteristics evaluation after an eight-hour empty stomach included: 1) history and physical examination; 2) blood pressure (BP); 3) fasting serum glucose, C-peptide (CP) and hemoglobin A1c (HbA1c); 4) fasting plasma lipids and 5) renal function parameters. Postprandial plasma glucose (PPG) were measured 2h after diet. Body mass index (BMI) was calculated as weight in kilograms divided by the square of height in meters. Systolic and diastolic BP values were the means of two physician-obtained measurements on the left arm of the seated participant. Serum total cholesterol (TC), triglyceride (TG), blood urea nitrogen (BUN), uric acid (UA), serum creatinine (SCr), CP levels were measured by an enzymatic method with a chemical analyzer (Hitachi 7600-020, Tokyo, Japan). Fasting plasma glucose (FPG) and PPG were quantified by the glucose oxidase-peroxidase procedure. HbA1c was measured by high-pressure liquid chromatography using an analyzer (HLC-723G7, Tosoh Corporation, Japan). The day-to-day and inter-assay coefficients of variation at the central laboratory in our hospital for all analyses were between 1% and 3%.

Definition Diabetes was defined as a self-reported history of physician-diagnosed T2DM or according to 1999 WHO criteria[18] as follows: fasting blood glucose (FBG) ≥7.0 mmol/L, or blood glucose ≥11.1 mmol/L 2h after an oral glucose tolerance test (OGTT), or random blood glucose ≥11.1 mmol/L. All the patients took the digital non-mydriatic fundus photography, and DR was diagnosed in a masked manner by independent ophthalmologists. Two independent ophthalmologists determined the presence of DR. Both eyes of each participant were photographed with a 45-degree 6.3-megapixel digital non-mydriatic camera (Canon CR6-45NM, Lake Success, NY, USA), repeated once only if necessary. The patients were classified into two groups (DR group and DWR group) according to the presence or absence of DR, regardless of its degrees of severity. The duration year was defined as the interval between the first diagnosis of diabetes and the time of enrollment in the present study. Age of onset year was the age at which an individual was diagnosed with T2DM for the first time. The clinical characteristics of participants are summarized in Table 1.

SNP Genotyping The genomic DNA was extracted from peripheral blood leukocytes by conventional phenol/ chloroform method. The genetic variant (rs10946398) of *CDKAL1* was genotyped using iPLEX (Sequenom, San Diego, CA, USA) with detection by the matrix-assisted laser desorption/ionization time-of-flight mass spectrometry platform. The DR and DWR groups were mixed for genotyping. The genotype distribution was in Hardy-Weinberg equilibrium (P>0.05), and there was a 99.9% genotype concordance rate when duplicated samples were compared across plates.

Statistical Analysis Kolmogorov-Smirnov Test was used detected whether continuous variables followed normal distribution. Variables that were not normally distributed were log-transformed to approximate normal distribution for analysis. Differences in variables between with-DR group and DWR group were determined by unpaired t-test. Between groups differences in qualitative traits, were accessed by χ^2 analysis. Age, sex, BMI, SBP, DBP, FPG, CP, PPG, TC, TG, HbAlc, BUN, Cr, UA, medical history, duration, age of onset, family history of DM, alcohol, rs10946398 allele, rs10946398 genotype were analyzed by Univariate logistic regression to estimate confounding factors possibly disturbing the relation of genetic variants to DR. We tested rs10946398 genotypic associations with DR risk using multivariate logistic regression (MLR) to adjust for a age, sex, BMI, SBP, FPG, PBG, TC, TG, HbA1c, duration, age of onset, family history of DM and alcohol. Odd ratios (ORs) with 95% confidence interval (95%CI) were assessed for the risk allele. In order to better investigate interaction between DR and rs10946398 of *CDKAL1*, we performed two analyses according to variable of allele and genotype of *CDKAL1*, respectively. OR with 95% CI were calculated for the relative risk of genetic variants of CDKAL1 with DR.

Table 1 Baseline characteristics of subjects

Demographical information	Total sample	DWR	DR	*P*
n	580	475	105	
Age (a)	64.73±10.85	64.77±10.96	64.54±10.33	0.781
Sex female (%)	362 (62.41)	299 (62.94)	63 (60.0)	0.247
Height (cm)	160.39±8.8	160.37±8.82	160.48±8.77	0.877
Weight (kg)	64.18±10.64	64.25±10.74	63.87±10.19	0.641
SBP (mm Hg)	138.76±20.9	138.21±21.25	141.2±19.12	0.061
DBP (mm Hg)	81.94±11.51	81.94±11.07	81.93±13.31	0.997
Blood plasma glucose profiles				
FPG (mmol/L)	8.69±3.11	8.34±2.75	10.28±4.01	<0.001
CP (mmol/L)	3.69±2.13	3.71±2.16	3.58±1.98	0.397
PPG (mmol/L)	14.76±5.71	14.01±5.37	18.13±5.98	<0.001
HbAlc (%)	7.18±1.57	6.97±1.4	8.12±1.92	<0.001
Lipids profiles (mmol/L)				
TC	5.36±1.11	5.35±1.09	5.37±1.18	0.849
TG	1.97±1.37	1.95±1.36	2.04±1.45	0.401
Renal function parameters				
BUN (mmol/L)	6.1±1.63	6.05±1.53	6.34±1.99	0.019
Cr (μmol/L)	67.39±22.33	66.72±20.05	70.43±30.46	0.029
UA (mmol/L)	0.29±0.08	0.29±0.08	0.28±0.08	0.059
Medical history				
Duration year (a)	7.38±6.2	7.09±6.13	8.67±6.39	0.001
Age of onset year (a)	57.57±10.79	57.91±10.8	56.04±10.62	0.023
CDKAL1 (rs10946398 C/A %)	265 (46.82)	55 (45.06)	210 (55.00)	0.021

SBP: Systolic blood pressure; DBP: Diastolic blood pressure; BUN: Blood urea nitrogen; UA: Uric acid; FPG: Fasting plasma glucose; PPG: Postprandial plasma glucose; TC: Total cholesterol; TG: Triglyceride; CP: C-peptide; Cr: Creatinine.

Results were analyzed using the Statistical Package for Social Sciences for Windows version 16.0 (SPSS, Chicago, IL, USA). Tests were two-sided and a P-value of <0.05 was considered significant.

RESULTS

Clinical Characteristics of Subjects In the present study, comparisons of baseline data between DR group and the DWR group were listed in Table 1. The DWR group included 176 males and 299 females (mean age, 64.77±10.96y) and DR group included 42 males and 63 females (mean age, 64.54±10.33y). DR group had significantly higher levels of FPG, PPG, HbAlc, BUN and Cr than those of DWR group (P<0.05 for all). Moreover, there were significantly longer duration and earlier onset of DM in DR group compared with DWR group (P<0.05 for all). Other variables of age, sex, height, weight, SBP, DBP, CP, TC, TG, UA was similar between the two groups (P>0.05 for all). The minor allele (C) frequency of rs10946398 was 45.06% and 55.00% in controls and cases, respectively. The percentage of DR was 14.95% and 20.75% in DR patients with A and C allele, respectively (Figure 1). The percentage of DR was 12.96%, 17.44% and 24.39% in diabetic patients with AA, CA and CC genotype, respectively (Figure 2).

Univariate Logistic Regression Analysis for Diabetes Univariate logistic regression models, including age, sex, BMI, hypertension, blood glucose profiles, lipid profiles,

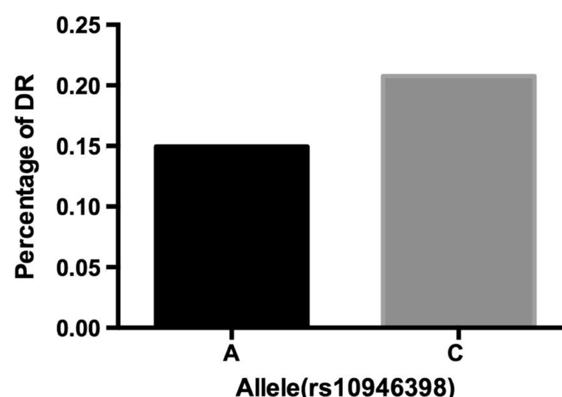

Figure 1 The prevalence of DR in two groups according to allele of rs10946398.

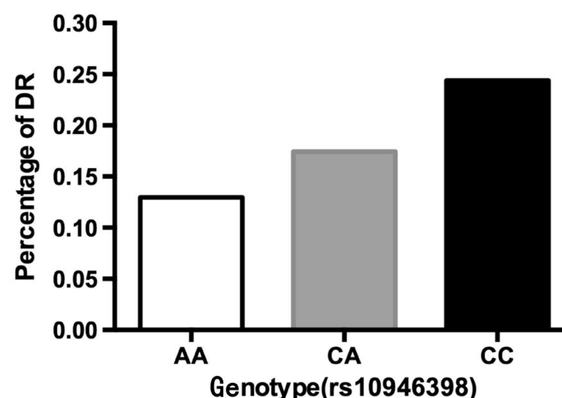

Figure 2 The prevalence of DR in three groups according to genotype of rs10946398.

Table 2 Univariate analysis for risk factors of DR

Variables	*β*	S.E.	*P*	OR	95%CI
Demographical parameters					
Age	-0.002	0.007	0.781	0.998	0.984-1.012
Sex	-0.119	0.156	0.446	0.888	0.654-1.206
BMI	-0.015	0.023	0.511	0.985	0.942-1.03
SBP	0.007	0.004	0.062	1.007	1.00-1.014
DBP	0.001	0.007	0.997	1.001	0.987-1.013
Blood glucose profiles					
FPG	0.171	0.023	<0.001	1.186	1.135-1.24
CP	-0.033	0.039	0.398	0.968	0.897-1.044
PPG	0.125	0.014	<0.001	1.133	1.103-1.165
HbAlc	0.407	0.046	<0.001	1.502	1.373-1.643
Lipids profiles					
TC	0.013	0.069	0.849	1.013	0.885-1.16
TG	0.044	0.052	0.402	1.045	0.943-1.158
Renal function parameters					
BUN	0.103	0.044	0.019	1.109	1.017-1.209
Cr	0.006	0.003	0.034	1.006	1.00-1.012
UA	-2.076	1.057	0.058	0.125	0.016-1.006
Medical history					
Duration	0.037	0.011	0.001	1.038	1.015-1.061
Age of onset	-0.016	0.007	0.023	0.984	0.971-0.998
Family history of DM	-0.201	0.16	0.209	0.818	0.598-1.119
Alcohol	-0.842	0.444	0.058	0.431	0.181-1.028
Gene information					
[1]rs10946398 C/A	0.398	0.168	0.024	1.488	1.071-2.069
[1]rs10946398 genotype	0.268	0.082	0.005	1.307	1.113-1.535

[1]*CDKAL1*; SBP: Systolic blood pressure; S.E.: Standard error; DBP: Diastolic blood pressure; BUN: Blood urea nitrogen; UA: Uric acid; FPG: Fasting plasma glucose; PPG: Postprandial plasma glucose; TC: Total cholesterol; TG: Triglyceride; CP: C-peptide; Cr: Creatinine.

Table 3 Multiple analysis for risk factors of DR

Variables	*β*	S.E	*P*	OR	95%CI
CDKAL1 (rs10946398 C/A)	0.309	0.126	0.043	1.362	1.021-1.887
CDKAL1 (rs10946398 genotype)	0.302	0.117	0.009	1.353	1.077-1.700

Adjusted for variables of age, sex, BMI, SBP, FPG, PBG, TC, TG, HbA1c, duration, age of onset, family history of DM and alcohol. S.E.: Standard error.

renal function parameters, medical history and SNP (rs10946398), were performed to determine the various clinical factors for the presence DR (Table 2). The results from univariate logistic models demonstrated that FPG, PPG, HbAlc, BUN, Cr, duration of DM, onset age of DM were significantly associated with DR (P<0.05 for all). In subjects with minor allele frequency (MAF) of rs10946398 in *CDKAL1*, the OR for DR was 1.488 for allele analysis (95% CI: 1.071-2.069, P=0.024) and 1.307 for genotype analysis (95% CI: 1.113-1.535, P=0.005) (Table 2).

Multiple Logistic Regression Analysis for Diabetes MLR demonstrated that genetic variant (rs10946398) of *CDKAL1* remained significant difference between case and control after adjustment for variables of age, sex, BMI, SBP, FPG, PBG, TC, TG, HbA1c, duration, age of onset, family history of T2DM and alcohol (P=0.043 for allele analysis and P= 0.009 for genotype analysis, Table 3). After adjusting for confounding factors, in subjects with MAF of rs10946398 in *CDKAL1*, the OR for DR was 1.362 for allele analysis (95% CI: 1.021-1.887, P=0.043) and 1.353 for genotype analysis (95%CI: 1.077-1.700, P=0.009) (Table 3).

DISCUSSION

We identified an association between rs10946398 of *CDKAL1* gene and DR in an independent case-control sample from Chinese Han population. To our knowledge, this study reported the first positive association between the rs10946398 of *CDKAL1* and DR in the Chinese Han population with an increased risk of 1.362 (95%CI: 1.021- 1.887 for genotype and P=0.043).

T2DM is known for its micro-vascular and macro-vascular

complications that contribute to high rates of mortality associated with this disease. DR is one of the most frequent micro-vascular complications known to be a leading cause of blindness, especially among working-age individuals [19-20]. The known risk factors such as duration of diabetes, level of glycaemic control, or concomitant vascular disease can't fully explain the substantial variation in the onset and severity of DR[21]. Therefore genetic factors play an important role in the susceptibility to T2DM and DR.

CDKAL1 gene is regarded as promising T2DM susceptibility gene involved in glucose regulation and insulin secretion/action identified by GWAS[22-24]. Replication studies reported significant associations between T2DM and rs10946398, rs7754840 and rs7756992 in the Chinese Han population [25], and rs10946398 and rs7754840 in the African American population [26]. An association of C allele of rs10946398 with T2DM has been replicated in the population of Asian, Caucasian, African, Arabs and Mexican population[27]. Association of *CDKAL1* variants (rs7756992, rs10946398) with low birth weight, an independent risk factor for T2DM, was reported by several studies [28-30]. Another Chinese study reported the association of the rs10946398 with cardiovascular risk but not with diabetic nephropathy[17].

Over 30 candidate genes involved in different metabolic mechanisms and functional pathways have been reported to be associated with DR [31]. In our present study, we focused on the relationship between rs10946398 (C allele) and DR among Chinese Han population to better evaluate the possible role of *CDKAL1* in the development of DR. Associations of other *CDKAL1* variants (rs10946398) with DR have been previously investigated in the Chinese population with no results [32]. In the current study, we found the rs10946398 (C allele) of *CDKAL1* conferred a high risk of DR (Table 3; Figure 1). One possible explanation for this discrepancy was the difference in the place of recruitment of subjects. In fact, the patients in the study by Fu *et al* [32] were recruited from the southwest of China (Sichuan Province, China) while in our study the T2DM patients were representative of the southeast population (the city of Shanghai). Gene SNPs had a different influence in T2DM for ethnic variation. Secondly, different examiners can also result in bias. But above all, when we compared DR with DWR groups, we used methods differed from theirs. In order to eliminate confounding factors which possibly disturbed the relationship between genetic variants and DR, evaluated by Univariate logistic regression (ULR), we performed MLR to control potential confounders for determining independent contribution of variables to DR. They found the relevant trend (OR>1, P>0.05) but failed to identify the association of rs10946398 of *CDKAL1* with DR in the Chinese population cause the potential confounders has not been removed. However, there is little evidence to demonstrate *CDKAL1* (rs10946398) to be an independent risk factor of DR. Such results might be attributed to the limited number of subjects that had insufficient statistical power to detect a slight effect of the common polymorphism in *CDKAL1* on DR susceptibility. A larger sample size, therefore, is necessary to detect the association between this *CDKAL1* genetic variant and T2DM.

In summary, we found the CC genotype or C allele of *CDKAL1* (rs10946398) as a genetic risk factor for DR among T2DM patients. However, a functional study, such as gene-targeting in mice, is needed to clarify the role of *CDKAL1* as a whole.

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