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Original article

CDKAL1 gene variants (rs7756992 and rs10946398) and susceptibility to type 2 diabetes: Evidence from a Jordanian case-control study

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Abstract. *Background:* Type 2 diabetes (T2D) is a prevalent metabolic condition characterized by chronic hyperglycaemia due to impaired insulin secretion or sensitivity. The CDKAL1 gene is associated with T2D risk because of its role in insulin biosynthesis, with SNPs such as rs7756992 and rs10946398 exhibiting variable associations across different populations. *Objective:* This study aims to assess the relationship between CDKAL1 variants rs7756992 and rs10946398 and the risk of T2D in the Jordanian population. *Method:* We conducted a case-control study involving 200 participants (100 with T2D and 100 matched controls) from Prince Hamza Hospital. Genomic DNA was extracted and analyzed for rs7756992 and rs10946398 using real-time PCR (Allelic Discrimination Assay). Clinical and genetic data were evaluated using SPSS and SNPStats software. *Results:* No significant association was found between rs7756992 and T2D or any clinical parameters. In contrast, significant differences in total cholesterol (TC) levels were identified between genotypes of rs10946398 within the T2D group (P =0.035) and in LDL cholesterol (LDL-C) levels within the control group (P =0.044). However, no significant association was observed between rs10946398 and T2D risk across all genetic models. *Conclusion:* The study revealed a significant association between the CDKAL1 rs10946398 variant and TC levels in T2D patients, indicating a potential gene-environment interaction. No overall significant associations with T2D risk were observed for either SNP. Further research with larger sample sizes is required to validate these findings in the Jordanian population. (www.actabiomedica.it)

Key words: CDKAL1, rs7756992, rs10946398, gene polymorphism, type 2 diabetes mellitus, genetic association, case-control study, jordan, susceptibility, risk factors

Introduction

Type 2 diabetes (T2D)

Type 2 diabetes (T2D) is a non-insulin-dependent metabolic disorder accounting for over 90% of the global prevalence of diabetes (1). Its prevalence has seen a rapid increase in recent decades across the globe, both within developed and developing nations. The prevalence has also been higher among Asians compared to Europeans (2). From 1990 to 2020, Jordan saw an increase in T2D prevalence from 14.0% to 16.0%, and it is projected to reach 20.6% by 2050 (3). T2D impairs insulin secretion from pancreatic β-cells or high blood glucose levels resulting from reduced tissue sensitivity to insulin (4). A combination of environmental factors, including unhealthy lifestyles, obesity, and genetic predisposition, contributes to its development (5). Gene polymorphisms impact proteins involved in insulin secretion and glucose metabolism, increasing the risk of T2D (6). Genome-wide association studies (GWAS) have identified over 500 loci associated with T2D, including genes like Tumor necrosis factor-alpha (*TNF-α*), solute carrier

family 30 member 8 (*SLC30A8*), transcription factor 7-like 2 (*TCF7L2*), and Cyclin-dependent kinase 5 (CDK5) regulatory subunit associated protein 1 like 1 (*CDKAL1*) (7).

CDKAL gene

CDKAL1 is a sixteen exons gene located on chromosome 6p22.3 (Figure 1); it spans 37 kb and encodes a 579 amino acids member of the methylthiotransferase family protein (8). It is abundant in mitochondria-rich tissues like the heart, skeletal muscles, pancreas, liver, and kidney (9, 10). *CDKAL1* is required for the appropriate posttranslational processing of pre-proinsulin to create mature insulin (11). it is vital for proper posttranslational modification of pre-proinsulin, which is necessary for the production of mature insulin. Dysregulation of *CDKAL1* impaired this process, leading to β-cell dysfunction and reduced insulin secretion (11, 12). Most abnormalities associated with *CDKAL1* SNPs were located in intron five of the *CDKAL1* gene (13). One of these is rs7756992, which leads to misfolded proinsulin, causing β-cell ER stress and destruction (11). This SNP is associated with increased T2D risk, as confirmed in European and East Asian populations (14-18). The *CDKAL1* rs10946398 variant also shows a strong correlation with T2D (19), with significant associations in studies from India and China (16, 20) but not in the United States (21).

So, underlying the significant role of *CDKAL1* and T2D development, this study aims to examine the association between the *CDKAL1* rs7756992 and rs10946398 variants and the risk of T2D in the Jordanian population by revealing the genetic influences of these variants on T2D within this group.

Material and methods

Study design and participants

In this case-control study, a total of 200 participants were included, comprising one hundred patients with T2D and one hundred age- and sex-matched controls. Patients with T2D were diagnosed by physicians and recruited from the Diabetes and Endocrinology Clinic at Prince Hamza Hospital between February 2023 and July 2023. The study received ethical approval from the Institutional Review Board (IRB) at the Hashemite University (2022, ref no. 2200724). All participants were fully briefed on the study's objectives and provided informed consent. The research adhered to Good Clinical Practice guidelines as stipulated by the Declaration of Helsinki (2013,Fortaleza).

Inclusion and exclusion criteria

The study included cases aged 33 to 66 who were diagnosed with T2D based on the American Diabetes Association (ADA) diagnostic criteria. Eligible patients had a confirmed diagnosis of diabetes with fasting blood glucose (FBG) levels ≥126 mg/dL and hemoglobin A1c (HbA1c) levels ≥6.5%. The control

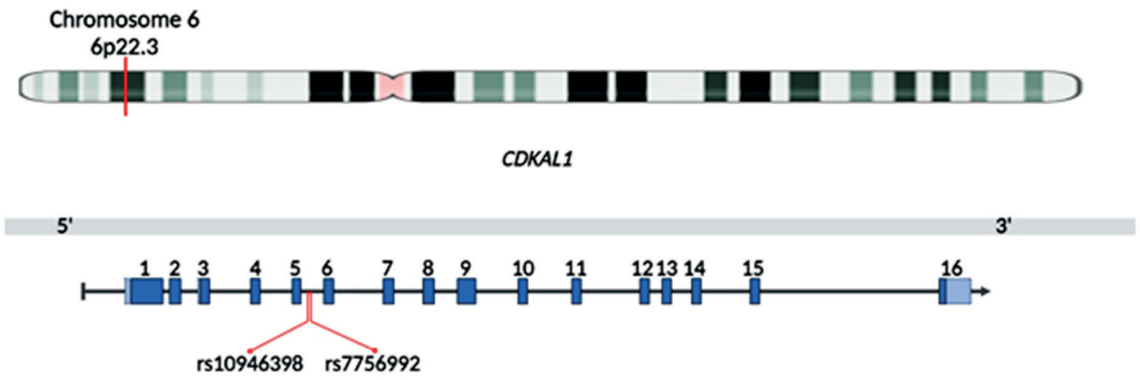

Figure 1. CDKAL1 gene with both rs7756992 and rs10946398 polymorphisms. rs7756992 and rs10946398 SNPs are located in intron five between exon 5 and 6 of the short arm p22.3 of chromosome 6.

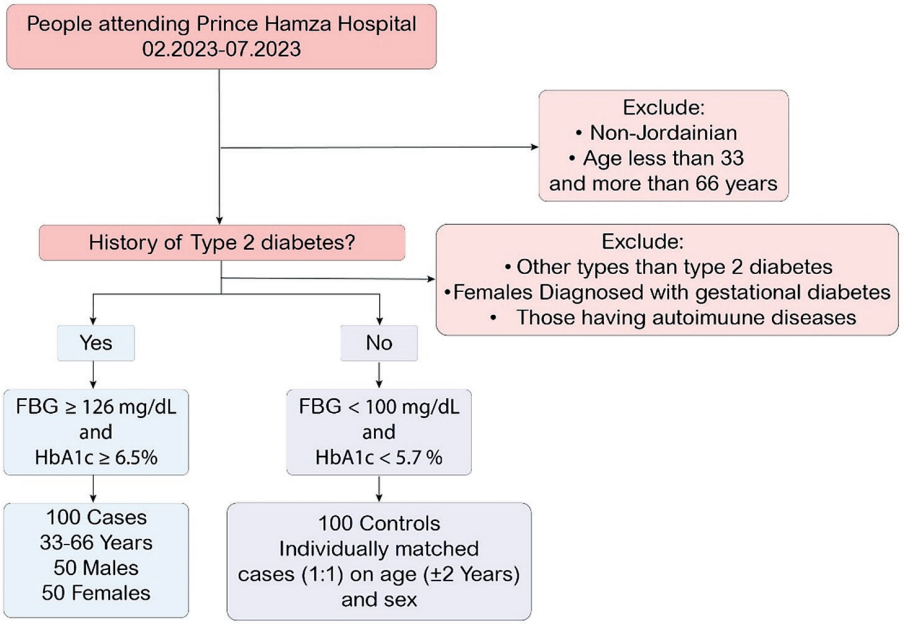

Figure 2. Inclusion and exclusion criteria chart.

group comprised Jordanians aged 33 to 66 years with no history of diabetes (type 1 or type 2), verified by HbA1c levels <5.7% and FBS levels <100 mg/dL. Participants younger than 33 years or with autoimmune diseases or other chronic diseases were excluded (Figure 2).

Sample collection

Two venous blood samples were collected from each participant for biochemical and molecular analyses. A plain tube was used for biochemical testing, while an ethylenediaminetetraacetic acid (EDTA) tube was used for HbA1c testing and DNA extraction. General chemistry tests and HbA1c measurements were performed using the Alinity c analyzer (Abbott, USA). HbA1c was determined by immunoassay technique, and FBG was determined by spectrophotometry method. HDL-C, LDL-C, TC, and TG lipid profiles were measured by the colorimetric method. In contrast, an LDL-C calculator was used to estimate VLDL-C using Martin's formula =TG/adjustable factor (22).The C-peptide assay was performed using the LIAISON® XL analyzer (Diasorin, Italy). The Homeostatic Model Assessment Calculator version 2.2.3 (HOMA2) was used to assess steady-state beta cell function (HOMA2-%B), insulin sensitivity (HOMA2-%S), and insulin resistance (HOMA2-IR) as relative percentages in comparison to a normal reference population using FBG and fasting C-peptide levels (23). Automatic calculations for these parameters were performed using the HOMA2 calculator (https://www2.dtu.ox.ac.uk/homacalculator/download.php).

DNA extraction and SNPs genotyping

Genomic DNA was extracted from peripheral blood samples using the QIAamp DNA Blood Mini Kit (QIAGEN, USA). The selection of SNPs was influenced by relevant literature on T2D obtained from the National Center for Biotechnology Information (NCBI) database (http://www.ncbi.nlm.nih.gov/). The listed primers and probes for the desired SNPs are shown in Table 1 (Macrogen, South Korea). Testing for *CDKAL1* polymorphisms using real-time PCR is feasible in most diagnostic laboratories equipped with molecular testing capabilities. However, availability is still limited in many primary care settings due to the high costs associated with genetic testing. Routine

Table 1. List of primers and probes for SNPs included in the study.

Gene/ polymorphism	Primer sequences 5'–3'	Fluorescent probe sequences 5'–3'	Annealing Temperature
CDKAL1 rs7756992	**Forward primer:** TTTGACAATTAATATTCCC **Reverse primer:** TTTTAACACACAAGAATC	**Probe A:** FAM-TGTATTTTAGTTTTAGATCTACAGTT-BHQ-1 **Probe G:** HEX-TGTATTTTAGTTTTGGATCTACAGTT-BHQ-1	54°C
CDKAL1 rs10946398	**Forward primer:** TATAATTAGGTTGAACTGGTT **Reverse primer:** GTAAGACAAGTGTTCTGATAT	**Probe A:** FAM- TTTAGTATCGTTATGCTGTCATTGC-BHQ-1 **Probe C:** HEX- TTTAGTATCGTTCTGCTGTCATTGC-BHQ-1	53°C

Abbreviations: FAM: Fluorescein Amidite, HEX: Hexachlorofluorescein, BHQ-1: Black Hole Quencher 1.

implementation remains a challenge due to these financial constraints.

Fluorescent signal detection during DNA amplification was performed using the AriaMx Real-Time PCR instrument (Agilent, USA). Fluorescein amidite (FAM) was used to detect the A allele, while hexachlorofluorescein (HEX) was used for the G allele, with both probes using Black Hole Quencher 1 (BHQ-1) as a fluorescence quencher. The reaction mixture for each qPCR sample contained 10 µL Promega GoTaq™ Sample qPCR Master Mix with Cyanine-X-Rhodamine (CXR) as a passive reference dye to normalize the fluorescence signal throughout the qPCR process (Promega, USA), 1 µL 10 µM primer and probes (Macrogen, South Korea), 10 µL nuclease-free water (Promega, USA) and 2 µL template DNA. The thermal cycling protocol began with an initial pre-denaturation step at 95°C for 12 minutes, followed by 40 cycles of denaturation at 95°C for 30 seconds, the annealing and extension steps were conducted at 54°C for rs7756992 and 53°C for rs10946398, each for 90 seconds.

Statistical analysis

Using version 26 of the Statistical Package for the Social Sciences (SPSS) software data management and statistical analyses were performed. Continuous variables were reported as means with standard deviations (SD), while categorical data were expressed as frequencies and percentages. Single and multiple logistic regression were utilized for conducting the association between the SNPs and T2D using the SNPStats software (https://www.snpstats.net/start.html). The correlation coefficient (r) was calculated using Pearson's method. The strength of the correlation was interpreted based on standard statistical guidelines *(24)*. The ANOVA F-test and Kruskal-Wallis H-test were used to evaluate differences in clinical variables, including FBG (mg/dL), HbA1c (%), TC (mg/dL), HDL-C (mg/dL), LDL-C (mg/dL), TG (mg/dL), VLDL-C (mg/dL), fasting C-peptide (ng/ml), and HOMA scores, in relation to the *CDKAL1* rs7756992 and rs10946398 variants. A *P* value of less than 0.05 was considered statistically significant.

Results

Demographic characteristics and clinical parameters

The study included a total of 200 participants (one hundred patients with T2D and one hundred controls) matched for age (30–70 years) and sex (50 men and 50 women). For patients with T2D and controls, the calculated mean age was 50.5 ± 8.1 years and 50.7 ± 7.8 years, respectively. Thirty percent of T2D patients had optimal glycemic control with

HbA1c levels below seven percent, whereas seventy percent of T2D patients had poor glycemic control. The majority of T2D patients (64%) were diagnosed in less than ten years, and 54 percent were between 50 and 66 years old. Eighty-six percent of T2D patients have a family history of diabetes. Sixty-two percent of patients smoked, and 34 percent were diagnosed with heart disease. A number of T2D patients used medications to regulate their blood pressure (38%) or lipid profile (18%). A summary of the demographic characteristics of both groups can be found in Table 2. A family history of T2D (P <0.001, r=2.5), heart disease (P <0.001, r=3.8) or smoking (P= 0.001, r=1.4) was highly correlated with T2D risk. The correlation and association between T2D and these three demographic characteristics are presented in Table 3.

Patients with T2D had significantly higher WC among women, BMI, SBP, and DBP (P < 0.05) than the control group. The parameters FBS, HbA1c, TG, C-peptide and insulin resistance increased significantly in patients with T2D compared to the control group (P <0.05). On the other hand, TC, LDL-C and the function and sensitivity of β-cells were significantly higher in controls than in patients with T2D (P < 0.05). WC in men, HDL-C and VLDL-C showed no difference between the two groups (P > 0.05). Table 4 lists the clinical characteristics for both groups as mean ± standard deviation.

Real-time PCR genotyping

The qPCR allelic discrimination assay results are shown in Figure 3.

The association between CDKAL1 rs7756992 and rs10946398 variants and T2D development

Both rs7756992 and rs10946398 allele frequencies showed no significant association with the presence of T2D (P > 0.05). The SNPStats website (https://www.snpstats.net/) was used to analyze the genotype differences between patients with T2D and control in codominant, dominant, recessive, and overdominant models within rs7756992 and rs10946398 SNPs after adjusting for age, sex, BMI, WC, family history of T2D, heart disease, hypertension, and smoking. In all genetic models, no significant difference was found in rs7756992 or rs10946398 genotypes between the control group and patients with T2D (P >0.05), as shown in Table 5.

Table 2. Demographic characteristics of patients with T2D and control.

	Control (n=100)	**Patients with T2D (n=100)**
Characteristics	**n (%)**	**n (%)**
Sex		
Male	50%	50%
Female	50%	50%
Age		
33-49	48%	46%
50-66	52%	54%
Maternal Status		
Single	12%	4%
Married	84%	86%
Divorced	2%	6%
Widowed	2%	4%
Glycemic control (HbA1c %)		
Optimal control (<7%)	100%	30%
Poor control (≥7%)	0%	70%
Duration of T2D (years)		
<10	-	64%
10-20	-	28%
≥20	-	8%
Having a family history of T2D		
Yes	38%	86%
No	62%	14%
Having heart disease		
Yes	2%	34%
No	98%	66%
Smoking		
Yes	42%	62%
No	58%	38%
Having lipid control medication		
Yes	4%	18%
No	96%	82%
Having hypertension control medication		
Yes	4%	38%
No	96%	62%

Abbreviations: T2D: Type 2 diabetes; %: percentage, n: number of samples.

Table 3. Association and correlation between demographic characteristics and T2D.

Demographic Characteristics T2D – Control (Ref)		*p*Ass - OR (95% CI) [a]	r(*p*cor) [b]
Having a family history of T2D	**No**	Ref	Ref
	Yes	**<0.001*** - 10.0 (3.8-26.8)	2.5 (**<0.001***)
Having heart disease	**No**	Ref	Ref
	Yes	**<0.001*** - 25.2 (3.2-198.9)	3.8 (**<0.001***)
Smoking	**No**	Ref	Ref
	Yes	**<0.001*** - 2.2 (1.0-5.0)	1.4 (**0.001***)

Abbreviations: OR: Odd Ratio, *p*Ass: *P* -value for the association, r: correlation coefficient, *p*cor: *P* -value for the correlation coefficient, ([a]) Chi-square, and ([b]) binary logistic regression were used. **P* <0.05 was considered as significant.

The comparison between gene polymorphism of CDKAL1 rs7756992 and rs10946398 genotypes and the investigated parameters

There was no significant difference between the rs7756992 genotype and any clinical characteristics (BMI, WC, FBG, HbA1c, TC, TG, HDL-C, LDL-C, VLDL-C, C-peptide, insulin resistance, β-cell function, and insulin sensitivity) among patients with T2D or controls (*P* >0.05), (Table 6). In contrast, there is a significant difference in TC level between AA (Median= 175.94, IQR=66.9) and CC (Median=211.73, IQR=43.3), *P* = 0.04 in T2D group and LDL-C between AC (149.55±39.7) and CC (104.83± 21), *P* = 0.04 in control group, (Table 7).

Table 4. Clinical parameters and Biochemical Characteristics of patients with T2D and control.

Clinical parameters and Biochemical characteristics		Control (n=100) (Mean ± SD)	Patients with T2D (n=100) (Mean ± SD)	*P*-value
BMI (Kg/m²)		27.2 ± 4.5	32.0 ± 6.1	**<0.001***[a]
WC (cm)	**Males**	97.3 ± 12.1	100.4 ± 18.2	**0.480** [a]
	Females	86.3 ± 13.6	96.6 ± 19.5	**0.036***[a]
SBP (mmHg)		129.6 ± 19.8	141.3 ± 24.3	**0.010***[a]
DBP (mmHg)		81.1 ±12.1	86.5 ± 11.7	**0.023** [a]
FBG (mg/dL)		93.8 ± 10.7	194.3 ± 99.9	**<0.001***[b]
HbA1c (%)		5.2 ± 0.3	9.1 ± 2.5	**<0.001***[b]
TC (mg/dL)		211.4 ± 41.25	196.8 ± 53.0	**0.030***[b]
TG (mg/dL)		139.6 ± 84.0	181.6 ± 115.0	**0.029***[b]
HDL-C (mg/dL)		46.9 ±10.1	44.4 ± 13.4	0.305 [a]
LDL-C (mg/dL)		138.2 ± 37.8	117.2 ± 40.4	**<0.008***[a]
VLDL-C (mg/dL)		25.9 ± 10.5	29.5 ± 12.5	0.105[b]
C-peptide (ng/dL)		2.5 ± 0.8	4.4 ± 3.3	**<0.001***[b]
HOMA2-IR		1.8 ± 0.6	4.4 ± 3.6	**<0.001***[b]
HOMA2-%B		134.3 ± 39.3	87.9 ± 68.1	**<0.001***[b]
HOMA2-%S		60.8 ± 20.8	36.5 ± 26.2	**<0.001***[b]

Clinical parameters for patients with T2D and control are presented as mean ± standard deviation (SD). *Abbreviations:* WC: Waist Circumference; SBP: Systolic Blood Pressure; DBP: Diastolic Blood Pressure; FBG: Fasting Blood Glucose: TC: Total Cholesterol; TG: Triglycerides; HDL-C: High-Density Lipoprotein Cholesterol; LDL-C: Low-Density Lipoprotein Cholesterol; VLDL-C: Very Low-Density Lipoprotein Cholesterol; C-peptide: Connecting peptide; HOMA2-IR: Homeostatic Model Assessment of Insulin resistance; HOMA2-%B: Homeostatic Model Assessment of β-cell function, HOMA2-%S: Homeostatic Model Assessment of Insulin sensitivity. *P* value obtained by ([a]) Independent samples *t*-test or ([b]) Mann Whitney *U*-test. **P* <0.05 was considered as significant.

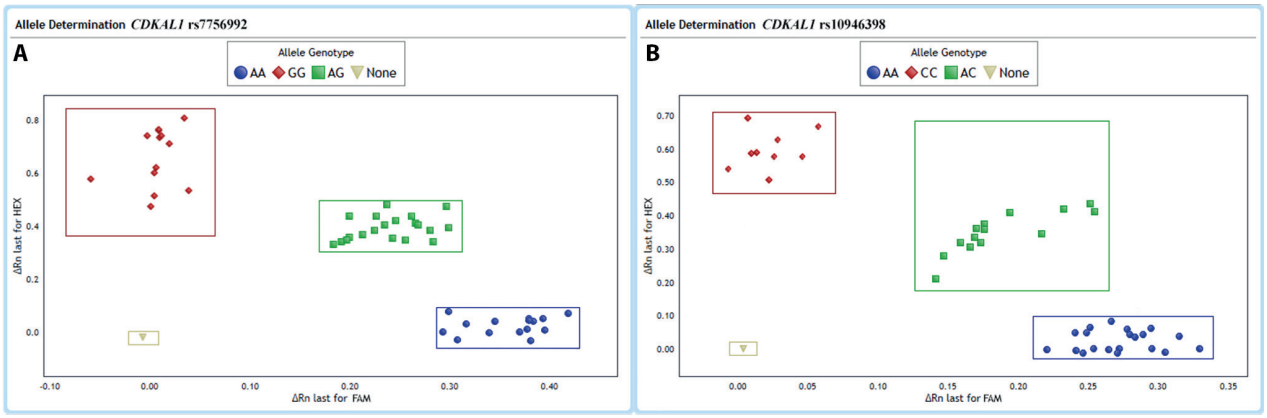

Figure 3. Results of the allele discrimination test using qPCR. The x-axis represents the delta-normalized reporter (ΔRn) for FAM, while ΔRn for HEX is represented by the y-axis, (a) *CDKAL1* rs7756992 as the genotypes are AA (blue circles), AG (green squares), GG (red diamonds), negative control (yellowish triangle), and positive control (within green squares)., and (b) rs10946398 as the genotypes are AA (blue circles), AC (green squares), CC (red diamonds), negative control (yellowish triangle), and positive control (within green squares).

Discussion

This study indicates that individuals with a family history of T2D are ten times more likely to develop the condition compared to those without such a history. This finding is consistent with research by Tremblay and Hamet, who highlight the genetic predisposition for T2D, where first-degree relatives of patients exhibit a substantially higher risk due to shared genetic and environmental factors (25). A strong association mirrors findings from studies which show that cardiovascular complications are the leading cause of mortality among T2D patients, with T2D nearly doubling the risk of coronary artery disease (26). Also, smoking was significantly more prevalent among T2D patients (62%) compared to the control group (42%), which exacerbates insulin resistance and increases the risk of T2D, as smoking contributes to the development of T2D through multiple biological pathways, including oxidative stress and chronic inflammation (27). Obesity is a well-established risk factor for T2D; our result is consistent with findings from Alkhalidy et al., which demonstrate that higher BMI is strongly associated with an increased risk of insulin resistance and subsequent T2D (28). Additionally central obesity, often measured by WC, is more closely related to insulin resistance and metabolic syndrome than BMI (29).This study supports the evidence that central obesity, particularly in women, plays a critical role in the development of T2D. We observe elevated blood pressure among patients with T2D than the control group, which is in line with research by Pavlou et al., who found that approximately two-thirds of patients with T2D also suffer from hypertension (30). The combination of high blood pressure and diabetes dramatically increases the risk of stroke and heart attacks (31). Elevated FBG and HbA1c are hallmark indicators of poor glycemic control in T2D (32). We found that 70% of T2D patients had poor glycemic control with HbA1C >7%, and 72.9% of them had heart diseases, which have been observed in a study by Boye et al. demonstrates that maintaining HbA1c levels below 7% is critical in minimizing the risk of long-term complications like retinopathy nephropathy and peripheral vascular disease (33). Dyslipidemia is a common feature in T2D patients; we found that patients with T2D had elevated TG-C levels, which is indicative of insulin resistance and metabolic syndrome (34). While the control group had higher LDL-C and TC levels compared to T2D, this may be due to the lipid control medications taken by patients in the diabetic clinic at Prince Hamzah Hospital. Identifying *CDKAL1* polymorphisms in patients with T2D might justify more intensive monitoring strategies, particularly in lipid management, given the observed associations with cholesterol levels. For non-diabetic patients, detecting

Table 5. Allele Frequency and genetic model association analysis between patients with T2D and control within rs7756992 and rs10946398.

Gene / SNP ID	Allele/ Genotype	Control (n=100) n (%)	Patients with T2D (n=100) n (%)	P value	OR (95% CI)
CDKAL1 **rs7756992**	**Allele frequency**				
	A	124 (62%)	117 (58%)	0.47	1.00
	G	76 (38 %)	83 (42%)		0.86 (0.58-1.29)
	Codominant model				
	AA	49 (49%)	43 (43%)	0.40	1
	AG	26 (26%)	31 (31%)		1.89 (0.73-4.92)
	GG	25 (25%)	26 (26%)		1.52 (0.53-4.33)
	Dominant model				
	AA	49 (49%)	43 (43%)	0.19	1
	AG+GG	51 (51%)	57 (57%)		1.72 (0.75-3.95)
	Recessive model				
	AA+AG	75 (75%)	74 (74%)	0.76	1
	GG	25 (25%)	26 (26%)		1.16 (0.44-3.06)
	Overdominant model				
	AA+GG	74 (74%)	69 (69%)	0.27	1
	AG	26 (26%)	31 (31%)		1.64 (0.68-3.96)
	Log-additive				
	-	-	-	0.33	1.29 (0.77-2.16)
CDKAL1 **rs10946398**	**Allele frequency**				
	A	136 (68%)	122 (61%)	0.14	1
	C	64 (32%)	78 (39%)		0.82 (0.63-1.07)
	Codominant model				
	AA	46 (46%)	34 (34%)	0.82	1
	AC	44 (44%)	54 (54%)		1.25 (0.35-4.50)
	CC	10 (10%)	12 (12%)		1.97 (0.23-16.54)
	Dominant model				
	AA	46 (46%)	34 (34%)	0.63	1
	AA+AC	54 (54%)	66 (66%)		1.35 (0.39-4.62)
	Recessive model				
	AA+AC	90 (90%)	88 (88%)	0.59	1
	CC	10 (10%)	12 (12%)		1.75 (0.23-13.16)
	Overdominant model				
	AA+CC	56 (56%)	46 (46%)	0.89	1
	AC	44 (44%)	54 (54%)		1.09 (0.33-3.64)
	Log-additive				
	-	-	-	0.54	1.34 (0.53-3.44)

P value was adjusted by age, sex, BMI, WC, family history of T2D, heart disease, hypertension, and smoking. *Abbreviations:* n: number of samples, T2D: type 2 diabetes, CI: Confidence Intervals.
*P <0.05 was considered statistically significant.

Table 6. The comparison between rs7756992 SNP and the clinical characteristics of patients with T2D and control.

CDKAL1 rs7756992		Control n=100		Patients with T2D n=100	
Clinical characteristics	**Genotype**	**Mean ± SD or Median (IQR)**	***P* value**	**Mean ± SD or Median (IQR)**	***P* value**
BMI	**AA**	28.1 ± 5.0	0.33[a]	51.0 (11.0)	0.28[b]
	AG	27.0 ± 4.9		55.5 (13.0)	
	GG	26.4 ± 4.3		47.0 (7.0)	
WC	**AA**	92.8 ± 14.1	0.15[a]	93.0 (22.0)	0.89[b]
	AG	90.7 ± 12.3		92.5 (23.0)	
	GG	86.3 ± 13.5		87.0 (18.0)	
FBG	**AA**	92.5 ± 10.4	0.92[a]	151.4 (127.7)	0.48[b]
	AG	90.9 ± 9.7		186.0 (110.1)	
	GG	93.4 ± 10.8		143.2 (86.2)	
HbA1c	**AA**	5.3 (0.4)	0.82[b]	8.0 (2.7)	0.57[b]
	AG	5.3 (0.3)		8.2 (2.1)	
	GG	5.2 (0.3)		7.7 (3.9)	
TC	**AA**	214.2 (44.4)	0.41[b]	194.1 (77.7)	0.95[b]
	AG	203.3 (64.5)		191.0 (34.8)	
	GG	189.9 (74.6)		192.3 (48.4)	
TG	**AA**	137.3 (96.8)	0.06[b]	159.4 (105.4)	0.90[b]
	AG	126.4 (97.9)		156.0 (163.9)	
	GG	84 (76.4)		156.8 (114.8)	
HDL-C	**AA**	45.2 (12.2)	0.33[b]	42.2 (20.0)	0.76[b]
	AG	45.0 (10.5)		42.9 (17.8)	
	GG	50.3 (14.8)		41.0 (23.3)	
LDL-C	**AA**	141.5 (50.1)	0.62[b]	105.6 (65.0)	0.78[b]
	AG	130.6 (58.5)		117.6 (42.5)	
	GG	124.8 (75.6)		109.8 (54.7)	
VLDL-C	**AA**	24.6 (14.4)	0.06[b]	28.7 (12.5)	0.90[b]
	AG	23.3 (13.4)		26.9 (17.4)	
	GG	18.7 (11.7)		26.3 (14.4)	
C-peptide	**AA**	2.4 (1.1)	0.62[b]	3.5 (2.3)	0.87[b]
	AG	2.1 (0.9)		3.5 (3.6)	
	GG	2.1 (1.0)		3.1 (1.9)	
HOMA2-IR	**AA**	1.6 (0.9)	0.63[b]	3.3 (2.8)	0.52[b]
	AG	1.5 (0.6)		3.5 (3.0)	
	GG	1.6 (0.8)		3.0 (1.9)	
HOMA2-%B	**AA**	131.7 (38.8)	0.25[b]	69.2 (75.1)	0.83[b]
	AG	131.1 (38.2)		76.9 (94.1)	
	GG	116.1 (26.6)		72.7 (97.2)	
HOMA2-%S	**AA**	60.7 (27.6)	0.63[b]	30.3 (26.0)	0.54[b]
	AG	65.4 (22.0)		28.5 (29.4)	
	GG	62.5 (27.7)		33.4 (29.0)	

Abbreviations: T2D: Type 2 diabetes, SD: standard deviation, IQR: Interquartile Range, BMI: Body Mass Index; WC: Waist Circumference; FBG: Fasting Blood Glucose; HbA1c: Glycosylated hemoglobin; TC: Total Cholesterol; TG: Triglycerides; HDL-C: High-Density Lipoprotein Cholesterol; LDL-C: Low-Density Lipoprotein Cholesterol; VLDL-C: Very Low-Density Lipoprotein Cholesterol; C-peptide: Connecting peptide; HOMA2-IR: Insulin resistance; HOMA2-%B: β-cell function, HOMA2-%S: Insulin sensitivity. The *P* value was obtained from (a) the analysis of variance (ANOVA) test or (b) the Kruskal Wallis test to determine the association between genotype and clinical characteristics. **P* <0.05 was considered statistically significant.

Table 7. The comparison between rs10946398 SNP and the clinical characteristics of patients with T2D and control.

CDKAL1 **rs10946398**		**Control n=100**		**T2D patient n=100**	
Clinical characteristics	**Genotype**	**Mean ± SD or Median (IQR)**	***P* value**	**Mean ± SD or Median (IQR)**	***P* value**
BMI	**AA**	27.4±4.5	0.94[a]	30.8±5.4	0.60[a]
	AC	27.1±5		32.45±6.8	
	CC	26.87±5		33.33±4.8	
WC	**AA**	92.43±11.3	0.71[a]	98±14.36	0.85[a]
	AC	92.23±14.24		99.6±20	
	CC	86.8±24		94.8±26.1	
FBG	**AA**	94.6±9.5	0.53[a]	150.3(127.5)	0.99[b]
	AC	94.2±11.9		183.8(113.5)	
	CC	88.6±11.5		139.64(184.1)	
HbA1c	**AA**	5.3(0.3)	0.88[b]	8(3.1)	0.93[b]
	AC	5.3(0.5)		8.7(3.8)	
	CC	5.3(0.6)		9.2(4.2)	
TC	**AA**	224.3(51.6)	0.05[b]	**175.94(66.9)**	**0.04**[*b]
	AC	222.26(57)		203.01(51.8)	
	CC	179.04(39.4)		**211.73(43.3)**	
TG	**AA**	118.7(80)	0.86[b]	141.71(80)	0.57[b]
	AC	119.23(103.3)		155.5(174.5)	
	CC	102.74(152.9)		182.427(112)	
HDL-C	**AA**	45.24(13.9)	0.85[b]	39.03±11.9	0.12[a]
	AC	45.1(15.2)		47.32±14.62	
	CC	50.3(14.5)		46.73±7.35	
LDL-C	**AA**	134.64±34.64	**0.04***	101.3±44.6	0.13[a]
	AC	**149.55±39.74**		124.6±36.6	
	CC	**104.83±21**		129±36.45	
VLDL-C	**AA**	23.6(11.7)	0.74[b]	25.97(11.96)	0.34[b]
	AC	22.44(16.88)		26.9(21.83)	
	CC	19.75(20.31)		32.26(11.55)	
C-peptide	**AA**	2.4(1.2)	0.71[b]	3.7(2.1)	0.89[b]
	AC	2.2(0.8)		3.28(2.4)	
	CC	2.1(1.5)		4.2(6.8)	
HOMA2-IR	**AA**	1.75(0.9)	0.75[b]	2.96(2.2)	0.90[b]
	AC	1.6(0.6)		3.43(3.2)	
	CC	1.55(1)		3.97(5.2)	
HOMA2-%B	**AA**	124.8(37.5)	0.91[b]	83.4(60.3)	0.85[b]
	AC	131.6(42.8)		66.7(89.4)	
	CC	116.1(118.1)		108.1(171.1)	
HOMA2-%S	**AA**	59.14±20.4	0.77[a]	33.8(19.8)	0.88[b]
	AC	61.62±20.82		29.2(35)	
	CC	64.5±19.11		25.6(37.1)	

Abbreviations: T2D: Type 2 diabetes, SD: standard deviation, IQR: Interquartile Range, BMI: Body Mass Index; WC: Waist Circumference; FBG: Fasting Blood Glucose; HbA1c: Glycosylated hemoglobin; TC: Total Cholesterol; TG: Triglycerides; HDL-C: High-Density Lipoprotein Cholesterol; LDL-C: Low-Density Lipoprotein Cholesterol; VLDL-C: Very Low-Density Lipoprotein Cholesterol; C-peptide: Connecting peptide; HOMA2-IR: Insulin resistance; HOMA2-%B: β-cell function, HOMA2-%S: Insulin sensitivity. The *P*value was obtained from (a) for analysis of variance (ANOVA) test or (b) the Kruskal Wallis test to determine the association between genotype and clinical characteristics. **P* <0.05 was considered statistically significant.

these variants may indicate an increased risk for future T2D development, suggesting more proactive lifestyle modifications or closer metabolic monitoring. High insulin resistance levels and low insulin sensitivity levels were found in patients with T2D compared to the control group, suggesting that the effect of lipid metabolites accumulation within the liver and skeletal muscle causes impaired insulin sensitivity (35). C-peptide reflects the function of β-cells (36). Our study identified significantly higher levels of C-peptide in patients with T2D despite exhibiting lower β-cell function. This observation aligns with the pathophysiological characteristics of T2D, where insulin resistance plays a central role. In the early stages of T2D, β-cells respond to the increasing insulin demand by secreting higher amounts of insulin to overcome peripheral insulin resistance. C-peptide, a byproduct of endogenous insulin production, thus remains elevated in many T2D patients, particularly during the initial phases of the disease (37). Extensive research has been conducted on T2D genetics, including genome-wide association studies to identify numerous genetic loci associated with T2D. This study investigated two SNPs, rs7756992 and rs10946398, within the *CDKAL1* gene in Jordanian T2D patients. The *CDKAL1* gene encodes a 1-like protein that is associated with the regulatory subunit of CDK5. CDK5 is a serine/threonine enzyme that inhibits insulin secretion, while inhibition of this enzyme increases insulin secretion (38). The study revealed a significant difference in TC levels between the AA and CC genotypes within the T2D group for the rs10946398 variant (P =0.035). This significant difference might be reflective of an underlying gene-environment interaction and dietary habits, similar to what has been observed in *Cdkal1* –/– mice models. In these mice, early-stage reductions in fat accumulation were evident but became less pronounced after prolonged high-fat feeding (39). This suggests that while genetic factors, such as variations in *CDKAL1*, can initially influence metabolic traits like lipid accumulation, prolonged exposure to a high-fat diet may attenuate these effects. Furthermore, ethnic-specific dietary patterns could play a role in the expression of genetic risk. The same research suggests that BMI can modulate *CDKAL1* variants associated with T2D risk through gene-environment interactions, where differences in dietary habits, such as a Western diet compared to an East Asian diet, might influence the extent to which these genetic variants affect metabolic outcomes, including cholesterol regulation (1). A similar contribution may be attributed to the significant differences observed in LDL levels between the AC and CC genotypes within the control group for the same variant. The observed TC and LDL-C differences in our study could thus represent a similar gene-environment interaction, where the effect of the rs10946398 variant on lipid metabolism is modified by dietary intake and possibly other environmental factors. Regardless of the significant role of *CDKAL1* in glucose metabolism, no significant association was found between rs7756992 and rs10946398 among T2D. However, based on a meta-analysis of twenty-one studies involving Caucasian, Asian, and African subgroups, *CDKAL1* gene rs7756992 A/G polymorphism was linked to T2D, as the G allele might be associated with T2D predisposition in Caucasians and Asians (P <0.05), but not among Africans (P >0.05) (8). Our study showed no significant association between T2D and rs7756992 SNP (P >0.05) in all models, which is consistent with findings from other populations, such as Norwegians (40) and Moroccans (41). Also, no statistically significant difference in rs7756992 (A, G) alleles was found between patients with T2D and the control group, similar to the results reported among Egyptians (42). Additionally, rs7756992 (AA, Ag, GG) genotypes showed no significant association with any of the clinical parameters (BMI, WC, FBG, HbA1c, TC, TG, HDL-C, LDL-C, VLDL-C, C-peptide; HOMA2-IR, HOMA2-%B, HOMA2-%S). For rs10946398, a meta-analysis of the *CDKAL1* rs10946398 locus across different ethnic groups and genetic models found significant associations between this polymorphism and T2D. In the Asian population, the dominant genetic model showed a significant association with T2D risk, while the allelic and recessive models did not show clear associations. In non-Asian populations, all genetic models demonstrated a significant link between the rs10946398 C/A variant and T2D risk (1). Another meta-analysis involving 13 studies from Europe, Asia, Africa, and Latin America found a significant association between the *CDKAL1*

rs10946398 C/A polymorphism and the risk of T2D. Using a dominant genetic model, individuals with the CC or CA genotype were at an increased risk of developing T2D, while those with the AA or CA genotype had a reduced risk compared to CC carriers (43). Our findings differ from those reported in previous studies, likely due to the relatively small sample size, which highlights one of the limitations of this research. In conclusion, Testing for *CDKAL1* polymorphisms may be indicated in patients with poorly controlled T2D, particularly those with a family history of diabetes or unexplained dyslipidemia. In non-diabetic patients, genetic testing could be considered in cases of significant family history or early metabolic syndrome to support preventive measures. Our study identified a significant association between the *CDKAL1* rs10946398 variant and TC levels in T2D patients‘, and in LDL-C levels among the control group, suggesting a potential gene-environment interaction similar to observations in animal models. Nonetheless, there is an absence of significant associations for both rs7756992 and rs10946398 across all genetic models, so further analysis is needed among the Jordanian population with a larger sample size.

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