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Single Nucleotide Polymorphisms in CDKAL1 Gene are not Associated with Risk of Type 2 Diabetes Mellitus in a Group of Egyptian Population

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Type 2 diabetes mellitus (T2DM) is a growing public health problem based on many causes, and its etiology still unclear. Genome-wide association (GWA) studies have revealed new single nucleotide polymorphisms (SNPs) that are associated with T2DM.? To evaluate the association between genetic polymorphisms of?CDKAL1?(rs7756992 and rs7754840) and the susceptibility to T2DM in a group of Egyptian patients. This case-control study included 75 patients with T2DM and 75 matched unrelated healthy controls. Genotyping was performed by using Real Time-PCR detected by Taqman probes. The frequency of genotypes, alleles, anthropometric measures, glycemic indices, HOMA-IR, and lipid profile was evaluated in patients and control. The odds ratios (ORs) and their 95% confidence interval (95% CI) were calculated by logistical regression to determine the associations between genotypes and T2DM.?Regarding rs7756992, the frequency of AA, AG, and GG genotypes was?45.3%, 41.3%, and 13.3% respectively in diabetic patients and 57.3%, 37.3%, and 5.3% respectively in healthy control (P = 0.151). Also, the frequency of A and G allele was not significantly different between patients and control (OR = 1.631, 95% CI = 0.985-2.702, P = 0.056). Similarly, genotypes CC, CG, and GG frequency and C and G alleles of rs7754840 were not significantly different between the two studied groups (P = 0.179 and 0.187) respectively. CDKAL1 (rs7756992 and rs7754840) were not associated with susceptibility to T2DM in the studied population. Moreover, there was no association between any of CDKAL1 genotypes with HOMA-IR or HbA1C in patients with diabetes.

Keywords: CDKAL1 Gene; Egyptian Population; PCR, Egyptian Population; Single Nucleotide Polymorphisms; Type 2 Diabetes.

Diabetes mellitus is a common health problem worldwide and its prevalence has been rising rapidly in both the developed and developing world[1]. It is expected that 591.9 million adults will have diabetes by 2035[2]. Type 2 diabetes mellitus (T2DM) is the most frequent form of diabetes. It is a multifactorial disease caused by a complex interplay of many environmental factors. However, it has been assumed that these factors can cause diabetes only in the presence of genetic susceptibility[3].

Genome-wide association (GWA) studies have revealed multiple genetic polymorphisms that are associated with T2DM. One of these is the polymorphisms of Cyclin-dependent kinase 5 regulatory subunit-associated protein 1 – like 1(CDKAL1)[4].

CDKAL1 is located on chromosome 6p22.3[4]. CDKAL1 is a 65-kD protein encoded by the CDKAL1 gene and was found to be implicated in beta cell dysfunction and T2DM susceptibility[5]. The exact mechanism by which CDKAL-1 modifies the insulin secretion in pancreatic beta cells is still unclear. One of the postulated mechanisms is the inhibition of CDK5 activation. CDK5 has been shown to blunt insulin secretion in response to glucose and to play a permissive role in the decrease of insulin gene expression that results from glucotoxicity, as well as in the pathophysiology of ß-cell dysfunction and predisposition to T2DM[6]. Thus, one can speculate that reduced expression of CDKAL1 would result in enhanced activity of CDK5 in ß cells, which would lead to decreased insulin secretion[6].

Association studies of CDKAL1 genetic variants with T2DM risk gave variable results in different ethnic populations. While some studies reported the association between CDKAL1 genetic variants and the risk of T2DM[7,8], other studies found no association[9,10]. To explore this issue, this study was done to evaluate the association of rs7756992 and rs7754840 SNPs within the CDKAL1 gene with T2DM susceptibility in a group of the Egyptian population.

Subjects and Methods

Subjects

This is study included 75 patients with T2DM (group 1) attending the Endocrinology and Diabetic Clinic at kasr al Ainy Hospitals, Cairo University and 75 normal unrelated age and sex matched subjects were selected as control (group 2).

Study Design

This is a case-control cross-sectional study. The subjects were enrolled from Endocrinology and Diabetic Clinic at kasr al Ainy Hospitals, Cairo University during the period between April 2018 to April 2019. The study protocol was approved by the Institutional Ethical Committee of Kasr Al Ainy Hospital. All patients and control subjects gave informed consent to participate in this study. The study protocol and procedures conform to the ethical guidelines of the 1975 declaration of Helsinki.

Subjects and Methods

The study comprised 150 subjects included 75 patients with T2DM and 75 healthy subjects. The presence of diabetes was identified based on the American Diabetes Association criteria. Patients between 40-70 years, having diabetes for more than 10 years, and receiving oral hypoglycemic drugs were included in the study. Patients with type 1diabetes and diabetes secondary to endocrinopathies, pancreatic diseases or drugs have been excluded.

All patients and control subjects were subjected to thorough medical assessment including determination of age, gender, blood pressure, anthropometric measures [weight, height and body mass index (BMI)]. Biochemical tests including fasting blood glucose, fasting insulin, glycosylated hemoglobin (HbA1c), total cholesterol, high-density lipoprotein cholesterol (HDL), Low-density lipoprotein cholesterol (LDL), and triglycerides were done. Insulin resistance status was determined using the HOMA-IR calculation, from the following equation: fasting insulin (microU/L) x fasting glucose (nmol/L)/22.5[11]. Recognition of CDKAL1 polymorphisms (rs7756992 and rs7754840) was done by real-time PCR.

Sample Collection and Biochemical Assay

Eight ml of blood were saved from each participant and split as follows: Two ml of plasma were extracted by aseptic venipuncture to pre-chilled EDTA vacutainer tubes for the genomic DNA test, DNA samples were preserves at -20°C to be used for TaqMan real-time PCR; Two ml of blood were drawn to EDTA tube for quantifying HbA1C; Two ml of blood were reserved in a red-topped serum separator tube, serum was collected by centrifugation, samples were examined for serum insulin and lipid profile, and Two ml of blood was extracted into fluoride tube for determining serum fasting glucose. Clinical chemistry investigation was done on Dimension RxL Max (Siemens, USA), while serum insulin was tested on Cobas e 411(Rosh diagnostics, Germany).

Isolation of Genomic DNA and SNP Genotyping

DNA extraction and analysis were tested using Gene JET Whole blood Genomic DNA Purification Mini Kit (Thermo Fisher, USA). DNA

quantification was tested by Qubit dsDNA BR assay kit 10 with the use of Qubit 2.0 fluorometer (Invitrogen, UK).

Genetic testing of TCF7L2 polymorphisms (rs 7930146, rs12255372) was evaluated using RT-PCR allelic discrimination assays through using Taq-Man SNP Genotyping Assays by using Step One Real-Time PCR systems (Applied Biosystems, Foster City, USA). The fluorescent dyes VIC and FAM were used to label the probes. A total of 25 µl volume containing 12.5 ul of TaqMan Universal PCR Master Mix (2x), 1.25 ul of TaqMan SNP Genotyping Assay (20x) and 20 ng of genomic DNA of PCR reactions was consumed. Cycling conditions were 95°C for 10 min, 50 cycles of 92°C for 10 s and 60°C for 1 min. The Applied Biosystems Real-Time PCR System software (Applied Biosystems, Foster City, USA) was used for allele discrimination.

Statistical Analysis

The statistical package SPSS version 23 was used for statistical analysis. For quantitative variables mean, standard deviation, median, minimum and maximum were used in data analysis. Frequencies (number of cases) and relative frequencies (percentages) were used for categorical variables. Unpaired t test was used to compare 2 groups and analysis of variance (ANOVA) was when comparing more than 2 groups. Chi-square (X2) test was performed to compare categorical data. When the expected frequency is less than 5, Exact test was applied instead .Genotype and allele frequencies were compared between the disease and the control groups using logistic regression. Odds ratio (OR) with 95% confidence intervals was calculated. P-values less than 0.05 were considered as statistically significant.

RESULTS

General Characteristics of the Study Subjects

The study included 150 subjects divided into 75 patients with T2DM and 75 age and sex-matched healthy control volunteers. Their age ranged from 40-70 years. A significant difference was identified in comparing BMI, waist circumference, blood pressure, fasting plasma glucose, fasting insulin, HOMA-IR, HbA1c, triglycerides, total cholesterol and LDL-cholesterol between the two studied groups (Table 1).

CDKAL1 rs7756992 Genotypes and Alleles in Diabetic Patients and Control

The frequency of rs7756992 genotypes in patients with diabetes was (AA: 45.3%; AG: 41.3%; GG: 13.3%), that was not significantly different from its frequency in non-diabetics (AA: 57.3%; AG: 37.3%; GG: 5.3). Also, there was no association of rs7756992 alleles (A, G) with T2DM (Table 2).

Table 1. Clinical and Laboratory parameters of diabetic patients and healthy subjects

Variable	Patients with T2DM n=75	Healthy subjects n=75	**P value*
Age (mean/years)	55.91± 7.77	53.92 ±10.28	0.184
Gender (male/female) (%)	44/56	44/56	1
[1]BMI(kg/m2)	31.21±5.85	26.86±3.09	<0.001
Waist circumference(cm)	108.87±10.25	101.93±8.06	<0.001
Fasting glucose (mg/dl)	180.88±65.52	85.21±8.35	<0.001
Fasting insulin (uU/mL)	25±20.77	5.7±1.95	<0.001
[2]HOMA-IR	12.21±13.16	1.22±0.48	<0.001
[3]HbA1c	8.63±1.9	5.3±0.4	<0.001
Triglygerides (mg/dL)	169.01±71.97	89.33±30.15	<0.001
Total Cholesterol (mg/dL	202.87±52.04	164.39±33.85	<0.001
[4]HDL-C (mg/dL)	37.87±9.42	38.47±10.37	0.711
[5]LDL-C(mg/dL)	134.41±41.63	113.25±30.29	<0.001

Values are mean ± SD. *P value <0.05 is considered significant.[1]BMI: body mass index; [2]HOMA-IR Homeostatic model assessment; [3]HbA1C: glycated hemoglobin; [4]HDL-C: high -density lipoprotein cholesterol; [5]LDL-C: low density lipoprotein cholesterol

CDKAL1 rs7754840 Genotypes and Alleles in the Studied Groups

The detected genotypes of rs7754840 in diabetic patients were CC (20%), CG (40%) and GG (40%), while in healthy subjects were CC (9.3%), GG (40%) and GG (31.7%). The frequencies of different genotypes did not significantly differ between both groups (Table 2).

Table 2. Frequencies and univariate analysis of SNP genotypes and alleles in the two group

[1]SNP	Genotype/ Allele	Patients with diabetes n=75	Healthy subjects n=75	OR (95% CI)	**P value*
rs7756992	AA (n=77)	34(45.3%)	43(57.3%)	1	0.151
	AG (n=59)	31(41.3%)	28(37.3%)	1.4(0.709-2.766)	
	GG (n=14)	10(13.3%)	4(5.3%)	3.162(0.912-10.967)	
	A allele	99(66%)	114(76%)	1	0.056
	G allele	51(34%)	36(24%)	1.631(0.985-2.702	
rs7754840	CC(n=22)	15(20%)	7(9.3%)	1	0.179
	CG(n=65)	30(40%)	35(46.7%)	0.4(0.144-1.111)	
	GG(n=63)	30(40%)	33(44%)	0.424(0.152-1.182)	
	C allele	60(40%)	49 (32.7%)	1	0.187
	G allele	90(60%)	101(67.3%)	0.728(0.454-1.167)	

Values are n (%). **P value <0.05* is considered significant
[1]SNP: Single-nucleotide polymorphism

Table 3. Clinical and laboratory data in diabetic patients according to different genotypes

	CDKAL1 rs7756992 genotypes			
variable	AA(n=34)	AG(n=31)	GG(n=10)	*P value*
[1]BMI	29.76±4.07	31.67±4.62	34.67±11.28	0.054
Waist circumference(cm)	108.26±8.29	108.23±9.27	112.9±17.46	0.415
Systolic B.P (mmHg)	132.94±18.99	127.1±14.19	142±25.73	0.076
Fasting glucose (mg/dl)	176.97±74.13	185.48±59.92	179.9±55.26	0.874
fasting insulin (uU/mL)	21±17.51	27.38±21.85	31.26±26.58	0.279
[2]HOMA-IR	10.81±14.05	12.75±10.91	15.3±16.89	0.617
[3]HbA1c (%)	8.52±2.02	8.69±1.88	8.78±1.71	0.903
Triglycerides (mg/dL)	189.68±86.6	145.45±50.73	171.8±54.55	0.044
Total Cholesterol (mg/dL)	195.5±50.35	206.48±49.87	216.7±64.94	0.470
[4]HDL-cholesterol (mg/dL)	36.03±7.02	39.9±10.35	37.8±12.83	0.257
	CDKAL1 rs7754840 genotypes			
variable	CC(n=15)	CG(n=30)	GG(n=30)	*P value*
[1]BMI	31.93±8.61	32.12±5.7	29.94±4.01	0.309
Waist circumference(cm)	109.47±14.29	109.4±10.96	108.03±6.95	0.851
Systolic B.P. (mmHg)	138.67±22.64	126.33±14.5	133.67±19.21	0.084
Fasting glucose (mg/dl)	181.67±54.91	186.23±69.17	175.13±68.21	0.809
fasting insulin (uU/mL)	34.1±22.71	25.04±23.79	20.42±14.88	0.114
[2]HOMA-IR	16.55±14.49	12.72±15.75	9.53±8.66	0.234
[3]HbA1c (%)	8.65±1.62	8.47±1.88	8.77±2.1	0.832
Triglycerides (mg/dL)	153.27±61.03	163.67±80.78	182.23±67.48	0.393
Total Cholesterol (mg/dL)	189.93±61.36	218.93±44.49	193.27±51.62	0.089
[4]HDL-cholesterol (mg/dL)	34.4±10.92	41±9.87	36.47±7.28	0.047

Values are mean ± SD. *P value <0.05 is considered significant.
[1]BMI: body mass index;[2]HOMA-IR Homeostatic model assessment;[3]HbA1c: glycated hemoglobin;[4]HDL-C: high-density lipoprotein cholesterol

The frequency of the C allele was not significantly different between diabetic patients (40 %) and healthy control (32.7%). Also; G allele frequency didn't differ between both groups and it was not associated with increased diabetes risk (OR = 0.728, 95% CI = 0.454-1.167, P = 0.187) (Table 2).

Clinical and Laboratory Data in Diabetic Patients According to Different Genotypes

A significant difference could be detected upon comparing triglycerides among the three genotypes of CDKAL1 rs7756992 (P=0.044). While no significant difference could be detected upon comparing BMI, waist circumference, systolic and diastolic blood pressure, fasting glucose, fasting insulin, HOMA-IR, HbA1c, total cholesterol, or HDL (P value= 0.054, 0.415, 0.076, 0.389, 0.874, 0.279, 0.617, 0.903, 0.470, 0.257 respectively) (Table 3).

A significant difference could be detected by comparing HDL cholesterol among the three genotypes CDKAL1 rs7754840 (p=0.047). While no significant difference could be detected upon comparing BMI, waist circumference, systolic and diastolic blood pressure, fasting glucose, fasting insulin, HOMA-IR, HbA1c, triglycerides, total cholesterol (p=0.309, 0.851, 0.084, 0.275, 0.809, 0.114, 0.234, 0.832, 0.393, 0.089 respectively) (Table 3).

DISCUSSION

An increase in the incidence of T2DM in Egypt has been observed recently, and hence successful management of the disease requires understanding the etiology of the condition. Different genetic variants have been studied and found to influence the incidence and progression of the disease. CDKAL1 gene was found to have a role in the regulation of insulin secretion through inhibition of CDK5 activation[12]. Thus, one can speculate that reduced expression of CDKAL1 would result in enhanced activity of CDK5 in ß cells, which would lead to decreased insulin secretion. In agreement with this assumption, this locus was significantly associated with small decreases in insulin response to a glucose load[13]. rs7756992 was found to be implicated in T2DM etiology in many human groups. A Chinese study included 1825 subjects with T2DM, 1487 with impaired glucose regulation and 2200 with normal glucose regulation; to investigate 17 SNPs that have been identified from Caucasians through GWAS. The study affirmed the associations of rs7756992 SNP in the CDKAL1 gene with the risk of impaired glucose regulation and T2DM[14]. Also, a Japanese study examined the association of 14 SNPs within 11 candidate loci with T2DM, showed an association of CDKAL1 rs7756992 with T2DM[15]. However, in the current study, no special pattern of association could be detected between rs7756992 gene polymorphisms and T2DM susceptibility and the frequency of rs7756992 (AA, AG, GG) genotypes in patients with diabetes was not significantly different from its frequency in non-diabetic subjects. Also, there was no statistically significant difference of rs7756992 alleles (A, G) between both groups. Moreover, in this study there was no significant difference in fasting glucose, fasting insulin, HOMA-IR, HbA1c, among the three genotypes of CDKAL1 rs7756992 which confirms the lack of association between CDKAL1 rs7756992 and T2DM in the studied population. Similarly, a study performed in the Han Chinese population found that rs7756992 is not confirmed to be associated with T2DM or impaired glucose tolerance[9]. Same results obtained in a Norwegian population[16].

In the Arabic population, a Tunisian study reported no association of rs7756992 of CDKAL1 with T2DM; however, they suggested that the rs7756992 of the CDKAL1 gene have a protective effect against diabetic nephropathy [12]. Similarly, a study by Benrahma including 250 unrelated Moroccan diabetic patients using TaqMan allelic discrimination assays to genotype three SNPs including rs7756992 found a complete lack of association between this polymorphism and T2DM[17]. Moreover, a study of an Egyptian population including 180 T2DM patients found a lack of association between rs7756992 and the risk of T2DM under any genetic model or allele[10].

All of these ndings suggest variability in the contribution of this variant to the risk of T2DM. This discrepancy was con rmed by a meta-analysis by conducted on Caucasian, Asian and African populations, involving 62,567 subjects from 21 separate studies. In the whole population, a significant association was found between CDKAL1 gene rs7756992 A/G polymorphism

and T2DM under allelic dominant and recessive. In the subgroup analysis, a significant association was found in Caucasians and Asians, while no significant association was detected in Africans under allelic. Only under the recessive genetic model, a significant association was found between them[18].

As regards CDKAL1 rs7754840, no significant difference could be detected between CC, CG and GG genotypes in our study. Also, no statistical significance was detected upon examining C and G allelic distribution. In addition, there was no significant difference in fasting glucose, fasting insulin, HOMA-IR, HbA1c, among the three genotypes of CDKAL1 rs7754840 which proves the lack of association between CDKAL1 rs7756992 and T2DM in the studied population. In accordance with this work a study of 1464 patients with T2DM and 1467 controls from Finland and Sweden, revealed that rs7754840 polymorphism does not affect T2DM[19].

In contrast with the present study, a study of the Iranian population showed meaningfully association between CDKAL1 rs7754840 and increased risk of T2DM[20]. The same finding was confirmed in patient with gestational diabetes[21].

This discrepancy between different population studies regarding the association between CDKAL1 rs7754840 SNP and T2DM was con rmed by a meta-analysis conducted on 33,149 T2DM patients and 36,992 controls from 21 independent studies. In the whole population, a significant relationship between the CDKAL1 rs7754840 G/C gene polymorphism and T2DM was observed under. In a subgroup analysis, a significant association was shown to exist in the Asian and Arabic populations under the allelic, But in the Mexican subgroup, no significant association between the CDKAL1 rs7754840 G/C gene polymorphism and T2DM[22].

Several studies investigated the association of both (rs7756992 and rs7754840) SNPs with the risk of T2DM with controversial results. In agreement with our results regarding the association of both SNPs with T2DM, a study of Pima Indians provided that no evidence of an association between any of these SNPs and T2DM[23]. In partial accordance with the current study, a study on different populations showed no association between rs7756992 and rs7754840 in the Moroccan population, one of French, or in the Austrian population. However, they found an association of both SNPs with T2DM in another set of French population[24]. In contrast with our study, research in Lebanese Arabs revealed the association of both rs7756992 and rs7754840 with T2DM[25].

A meta-analysis of 21 studies for rs7756992 and 17 studies for rs7754840 variants of the CDKAL1 gene to evaluate the effect of CDKAL1 on genetic susceptibility for T2DM concluded that there are significant associations between CDKAL1 polymorphisms and type 2 diabetes but these associations vary in different ethnic populations[13].

Though the sample size was calculated before the start of this work, the discrepancy in the results may be due to several reasons including different ethnicity, study design, and epigenetics. More extensive, well designed with a larger number of patients and more powerful meta-analysis should be considered to detect the potential association of CDKAL1 gene polymorphism and T2DM.

CONCLUSION

In conclusion, the results of the present study suggest that CDKAL1 (rs7756992 & rs7754840) SNPs do not influence the susceptibility of T2DM in the studied group of the Egyptian population. There is no difference between diabetic and healthy subjects in the frequency CDKAL1 (rs7756992 & rs7754840) genotypes or alleles. Moreover, there was no association between any of CDKAL1 (rs7756992 & rs7754840) genotypes with BMI, HOMA-IR or HbA1C in patients with diabetes.

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