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ORIGINAL ARTICLE

Common variants in TCF7L2 and CDKAL1 genes and risk of type 2 diabetes mellitus in Egyptians

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Abstract In this work we studied association of common variants in transcription factor 7-like 2 (*TCF7L2*) and cyclin-dependent kinase 5 regulatory subunit-associated protein 1-like 1 (*CDKAL1*) genes with type 2 diabetes mellitus (T2DM) in Egyptians.

Subjects and methods: This is a case–control study; 180 T2DM patients and 210 control subjects were genotyped for *TCF7L2* rs7903146 and rs12255372 and *CDKAL1* rs7756992 single nucleotide polymorphisms (SNPs) by TaqMan method on real time polymerase chain reaction system (real time-PCR).

Results: *TCF7L2* rs12255372 and rs7903146 associated with T2DM ($p = 0.0001$ and 0.003; respectively). The rs12255372 variant T allele associated with 2-fold increased risk for T2DM and TT genotype carriers were at 3.58-folds higher risk to develop T2DM than wild genotype (GG) carriers. Meanwhile, rs7903146 variant T allele associated with 1.6-fold increased risk for T2DM and TT genotype carriers were at 2.3-folds higher risk than wild genotype (CC) carriers. Both *TCF7L2* SNPs significantly associated with T2DM under additive and dominant models and after adjustment for other covariates. On the other hand, *CDKAL1* rs7756992 showed no significant association with T2DM under any genetic model. Both *TCF7L2* SNPs were in strong LD ($P = 0.02$; $D' = 0.85$). Taking common *TCF7L2* rs12255372/rs7903146 GC haplotype as reference, multivariate analysis confirmed the association of rs12255372 T allele-containing haplotypes (TC and TT) with T2DM. Haplotype TC associated with 6.32 times-higher risk for T2DM (95% CI = 0.55–76.17, Pc = 0.04) followed by haplotype TT which associated with 3.88 times-higher risk for the disease (95%CI = 1.09–13.76, Pc = 0.03).

Conclusion: *TCF7L2* rs12255372 and rs7903146 common variants associate with T2DM risk in Egyptians.

1. Introduction

Type 2 diabetes mellitus (T2DM) is a metabolic disorder caused by decreased insulin sensitivity and impaired insulin secretion due to pancreatic beta cell defect [1]. In addition to

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environmental and lifestyle risk factors, genetic factors play an important role in disease pathogenesis [2]. So, identification of genetic architecture of T2DM is of great interest for risk prediction and preventive interventions.

T2DM represents a global major healthcare burden [3]. According to the International Diabetes Federation (IDF), Egypt is in the world 8th place in terms of diabetes incidence, affecting up to 9.3% of population, and due to a rapidly increasing and aging population, Egypt will have the highest number of people with diabetes in the region by 2025 [4].

According to genome-wide association studies (GWAS) and meta-analysis; more than 50 gene variants were identified to be associated with T2DM. Two common variants in transcription factor 7-like 2 (*TCF7L2*) gene on chromosome 10q25.3 have brought the most attention and were reported as the strongest genetic risk factor for T2DM [5]; a C-to-T substitution in intron 3 (IVS3C > T, rs7903146) [6–8] and a G-to-T substitution in intron 4 (IVS4G > T, rs12255372) [6,9]. The exact mechanism through which *TCF7L2* variants affect the risk for T2DM is still unclear. It has been postulated that *TCF7L2* gene variants may indirectly alter glucagon-like peptide 1 (GLP-1) levels, an insulinotropic hormone which plays a critical role in blood glucose homeostasis [10].

One of the loci most consistently associated with T2DM risk is the intronic variant within the cyclin-dependent kinase 5 regulatory subunit-associated protein 1-like 1 (*CDKAL1*) gene; A-to-G substitution in intron 5 (IVS5A > G, rs7756992) [11].

CDKAL1 gene, located on chromosome 6p22.3, encodes a 65-kD protein (*CDKAL1*) which is implicated in beta cell dysfunction and T2DM susceptibility [12]. *CDKAL1* might regulate insulin secretion induced by cyclin-dependent kinase 5 (CDK5) by binding to the CDK5 activator p35 [13–15]. It was postulated that down regulation of CDKAL1 expression might increase the activity of CDK5 [16]. However, the exact pathogenesis of how CDKAL-1 modulates insulin release in pancreatic beta cells and the susceptibility to T2DM by interactions between these proteins still need more investigations [17].

Association studies of these genetic variants with T2DM risk gave inconsistent results in populations from different ethnic origins, which have been attributed to ethnic variations, linkage disequilibrium pattern, as well as other non-genetic factors [18]. This is the first study to investigate the association of *TCF7L2* rs7903146 and rs12255372 and *CDKAL1* rs7756992 variants with T2DM risk in Egyptians.

2. Subjects and methods

2.1. Subjects

A total of 390 subjects, including 180 unrelated T2DM patients and 210 healthy controls, were enrolled in the current study. T2DM patients were recruited from the outpatient clinic of the National Diabetes & Endocrinology Institute. Diagnosis of diabetes based on the diagnostic criteria of the American Diabetes Association 2014 [19], i.e. fasting plasma glucose (FPG) $\geqslant$ 126 mg/dL or 2 h plasma glucose (PPG) $\geqslant$ 200 mg/dl or random plasma glucose (RBG) $\geqslant$ 200 mg/dl. Exclusion criteria were type 1 diabetes (T1DM), maturity onset diabetes of the young (MODY), or type 2 diabetes diagnosed before the age of 30 years.

Control subjects had normal glucose tolerance confirmed by fasting plasma glucose (FPG) < 126 mg/dl or 2 h plasma glucose (PPG) < 200 mg/dl or random plasma glucose (random blood sugar) (RBG) < 200 mg/dl, and no first-degree family history of diabetes. Informed consent was obtained from all subjects and the study protocol was approved by the Ethics Committee of the National Research Centre.

Clinical examination was applied including measurement of systolic blood pressure (SBP) and diastolic blood pressure (DBP). Anthropometric measurements (weight and height) were collected and used for BMI calculation according to the standard formula BMI = weight (kg)/[height (m)]2.

2.2. Biochemical analysis

Blood samples for biochemical screening tests were obtained from all subjects after a 12 h overnight fast. FPG, TC (total cholesterol), TG (triglycerides), HDL-C (high density lipoprotein cholesterol), LDL-C (low density lipoprotein cholesterol) and HbA1c (glycated Hb) were assayed on Cobas c311 auto analyzer (Roche Diagnostics, Germany).

2.3. Genotyping analysis

Genomic DNA was extracted from peripheral blood using QIAamp DNA extraction kit (Qiagen Hilden, Germany) according to the manufacturer's protocol. *TCF7L2* gene SNP rs7903146 (assay ID: C_29347861_10) and SNP rs12255372 (assay ID: C_291484_20) and *CDKAL1* SNP rs7756992 (assay ID: C_2504058_20) were genotyped by TaqMan allelic discrimination method on ABI 7500 real-time PCR system (Applied Biosystems, Foster City, CA). All primers and probes were designed by Applied Biosystems (Foster City, CA). For genotyping quality control, 10% of samples were randomly selected and measured in duplicates and the concordance rate was 100%.

2.4. Statistical analysis

Data were analyzed using SPSS version 16.0 for Windows (Chicago, IL, USA). Data were expressed as mean ± SD for continuous variables and as percentages of total for categorical variables. Intergroup significance was assessed by Student's *t*-test for continuous variables and χ^2 test for categorical variables. The Hardy–Weinberg equilibrium was estimated by χ^2 test. Chi-square was used to test the difference in alleles and genotypes frequency between groups. The Bonferroni correction method was applied for multiple testing. Associations of genotypes and alleles with T2DM were evaluated by logistic regression after adjustment for other covariates. Odds ratios (ORs), 95% confidential intervals (CIs) and *P* values were calculated. *P* value less than 0.05 was considered significant.

3. Results

3.1. General characteristics of the study subjects

The study included 390 subjects classified into 180 patients with T2DM and 210 control subjects. Their age ranged from 40 to 60 (years). Clinical and biochemical data are shown in

Table 1. Significant differences between patients and controls were identified in gender, BMI, SBP, DBP and lipid profile parameters; accordingly, those were the potential covariates that were controlled for in the subsequent analysis.

3.2. Association studies

Significantly higher minor allele frequency (MAF) of *TCF7L2* rs12255372 and rs7903146 were identified in T2DM patients ($p = 0.0001$ and 0.003, respectively). While MAF of *CDKAL1* rs7756992 showed no significant difference between patients with T2DM and control subjects ($p = 0.2$) (Table 2). Both *TCF7L2* SNPs significantly associated with T2DM under additive and dominant models, but not the recessive model. In contrast, *CDKAL1* rs7756992 did not show association with T2DM under any genetic model. Table 3 summarizes the association studies of these genetic variants with T2DM under different models.

3.3. Haplotype analysis

Both *TCF7L2* SNPs were in strong LD ($P = 0.02$; $D' = 0.85$). Taking the common *TCF7L2* rs12255372/rs7903146 GC haplotype as reference (OR = 1.0), multivariate analysis confirmed the association of rs12255372T allele-containing haplotypes (TT, TC) with T2DM. TT haplotype carriers were 3.88 times-at risk for T2DM (OR = 3.88, 95%CI = 1.09–13.76, Pc = 0.03), while TC haplotype carriers were 6.32 times-at risk for T2DM (OR = 6.3295%CI = 0.55–76.17, Pc = 0.04) (Table 4).

4. Discussion

The association between *TCF7L2* rs7903146 and rs12255372 and *CDKAL1* rs7756992 SNPs and T2DM susceptibility has been widely studied in different populations [5,14,20,21]. However, studies in different ethnic groups gave inconsistent results, while some replication studies confirmed this association, other studies failed to find significant association, proposing an ethnic variability.

TCF7L2 gene variants have been replicated in many ethnic groups and were associated with T2DM [5], including Caucasians [22], Europeans [23], Indians [24], Africans [20], and East Asians [25,26]. Among which, rs7903146 and rs12255372 SNPs showed the strongest association with disease susceptibility [5,6,20–26]. In Arabs, association between *TCF7L2* variants and T2DM varied, while strong association was demonstrated in Tunisians [27], Moroccans [5], Omanis [28] and Palestinians [29], weak or no significant association was reported in United Arab Emirati [30] or Saudi Arabians [31].

This is the first study to examine the association of these SNPs with T2DM in Egyptians. Our results extend the list of populations in which *TCF7L2* rs12255372 and rs7903146 are associated with T2DM. The strongest association was with *TCF7L2* rs12255372 ($p = 0.0001$) followed by rs7903146 ($p = 0.003$). The rs12255372T allele associated with 2-fold increased risk of T2DM and TT genotype increased the risk by 3.58-folds when compared with GG genotype. Meanwhile, rs7903146T allele associated with 1.6-fold increased risk while TT genotype increased the risk of disease by 2.3-fold when compared to CC genotype. Both SNPs were significant under additive and dominant models, but not the recessive model which might be attributed to the small sample size and low frequency of genotypes homozygous for the variant alleles in our sample.

In Tunisians, rs7903146T allele associated with increased susceptibility to T2DM and TT genotype carriers were at 56% higher risk of T2DM than CC genotype carriers [27], while in United Arab Emirati subjects, T2DM associated with rs12255372T variant but not rs7903146T variant with no association with metabolic syndrome, beta cell function or insulin resistance [30]. In Palestinians, rs7903146T variant associated with a 3.34 times higher risk of T2DM and TT genotype associated with an earlier onset of disease [29].

In a large meta-analysis, including data from 36 genetic association studies with 35,843 patients with T2DM and 39,123 controls, an association between *TCF7L2* gene and T2DM has been demonstrated. In which, rs7903146 CT genotype associated with a 1.4-fold higher risk for T2DM and TT genotype with a 2-fold increased risk when compared with wild genotype. The rs12255372 GT genotype associated with 1.4-fold increased risk of T2DM and TT genotype with a 1.9-fold higher risk for T2DM when compared with wild genotype [32].

In our patients, we could not replicate the association of *CDKAL1* rs7756992 with T2DM. *CDKAL1* rs7756992 has been associated with T2DM and functional studies indicated its relationship to impaired insulin secretion [33] by binding to the CDK5 activator p35 [13].

Our results were consistent with previous studies [21,34,35]. However, in a study on Lebanese population, rs7756992 associated with T2DM and the risk estimate conferred by the variant G allele was 1.3 [18].

A meta-analysis involved 62,567 subjects from 21 studies demonstrated a significant association between rs7756992 and T2DM. In the subgroup analysis, significant association was found in Caucasians and Asians, while no significant association was detected in Africans [36]. In another meta-analysis

Table 1 Demographic and biochemical characteristics of the study population.

Characteristic	Controls ($n = 210$)	T2DM ($n = 180$)
Age (Years)	53.40 ± 5.2	55.3 ± 6.1
Gender (male/female)	112/98	115/65*
BMI (kg/m^2)	24.65 ± 3. 21	27.31 ± 4.74*
Diabetes duration (Years)	–	7.9 ± 4.5
SBP (mm/Hg)	118.4 ± 11.2	132.6 ± 18.5*
DBP (mm/Hg)	79.6 ± 14.9	83.7 ± 13.2*
FPG (mg/dL)	86.16 ± 13.92	152.8 ± 14.5*
HbA1c (%)	4.9 ± 0.36	6.51 ± 1.21*
Triglycerides (mg/dL)	99.56 ± 34.18	127.6 ± 28.2*
TC (mg/dL)	182.7 ± 29.09	190.1 ± 41.56*
LDL-C (mg/dL)	104.7 ± 25.85	127.22 ± 31.9*
HDL-C (mg/dL)	51.9 ± 8.7	47.9 ± 10.2*

BMI: body mass index; SBP: systolic blood pressure; DBP: diastolic blood pressure; FPG: fasting plasma glucose; HbA1c: glycated Hb; TG: triglycerides; TC: total cholesterol; LDL-C: low density lipoprotein cholesterol; HDL-C: high density lipoprotein cholesterol.

* Significant *p*.

Table 2 Association of TCF7L2 and CDKAL1 SNPs with T2DM.

Gene	SNP	Cases MAF	Controls MAF	*P*	OR (95%CI)
TCF7L2	rs7903146	0.38	0.24	0.003	1.62 (1.17–2.25)
	rs12255372	0.37	0.17	0.0001	2.06 (1.45–2.93)
CDKAL1	rs7756992	0.40	0.45	0.2	0.81 (0.60–1.11)

MAF: minor allele frequency (defined based on frequency in controls).

Table 3 Association of TCF7L2 and CDKAL1 gene variants with T2DM risk under different models.

SNP	Genotype distribution[a]		Additive model		Dominant model		Recessive model	
	Controls $N = 210$	Patients $N = 180$	OR (95%CI)	*P*	OR (95%CI)	*P*	OR (95%CI)	*P*
TCF7L2								
rs7903146								
C/C	112 (53.3)	48 (26.6)	1.0 (Reference)					
C/T	95 (45.2)	126 (70)	3.09 (2.01–4.75)	< 0.00001	3.14(2.04–4.81)	< 0.0001	2.37 (0.58–9.65)	0.22
T/T	3 (1.5)	6 (3.4)	4.6 (1.12–19.43)		C/C vs. C/T + T/T		C/C + C/T vs. T/T	
rs12255372								
G/G	139(66.2)	60 (33.3)	1.0 (Reference)					
G/T	69 (32.9)	114 (63.4)	4.05 (2.65–6.2)	< 0.00001	3.9 (2.56–5.96)	< 0.0001	3.58 (0.71–17.99)	0.12
T/T	2 (0.9)	6 (3.3)	4.65 (1.12–19.14)		G/G vs. G/T + T/T		G/G + G/T vs. T/T	
CDKAL1								
rs7756992								
A/A	63 (30)	60 (33.3)	1.0 (Reference)					
A/G	105 (50)	96 (53.3)	0.96 (0.61–1.5)	0.21	0.85 (0.55–1.31)	0.48	0.61 (0.35–1.06)	0.08
G/G	42 (20)	24 (13.4)	0.6 (0.32–1.1)		A/A vs. A/G + G/G		A/A + A/G vs. G/G	

[a] Data shown as number of subjects (frequency).

Table 4 TCF7L2 rs12255372/rs7903146 haplotype distribution in patients and controls.

Haplotype	Controls (%)	Patients (%)	Pc	OR (95%CI)
G C	50	20	< 0.00001	1.0 (reference)
T T	38.5	50	0.03	3.88 (1.09–13.76)
T C	0	10	0.04	6.32 (0.55–76.17)
G T	11.5	20	0.07	4.66 (0.86–25.13)

Data were calculated by multivariate analysis.
Pc: corrected *p* after applying Bonferroni correction.

of 27 studies on *CDKAL1* rs7756992 involving 28,383 T2DM patients and 47,635 controls, the overall risk estimate for T2DM conferred by rs7756992G allele was 1.2 [37]. In a study investigating the association between T2DM risk variants and reduced birth weight in Chinese Han individuals, among 12 genetic variants, *CDKAL1* rs7756992 associated with increased risk of impaired glucose metabolism, low birth weight and decreased insulin secretion index later in life [17].

In conclusion, *TCF7L2* rs12255372 and rs7903146 common variants associate with T2DM susceptibility in Egyptians; however, we could not identify such association for *CDKAL1* rs7756992 variant in our patients.

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