A possible association between rs7903146 (C/T) polymorphism of the transcription factor 7-like 2 gene and type 2 diabetes mellitus in Egyptians

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Background
Type 2 diabetes mellitus (T2DM) is a very common polygenic metabolic disorder. Single-nucleotide polymorphisms of the transcription factor 7-like 2 (*TCF7L2*) gene have been reported to affect T2DM susceptibility by affecting insulin secretion and via its involvement in the Wnt signaling pathway.
Patients and methods
The present study investigated the association of rs7903146(C/T) polymorphism of the *TCF7L2* gene with T2DM. The study involved 42 Egyptian adults who were diagnosed with T2DM and 42 healthy adults taken as controls.
Allele-specific PCR was performed to detect *TCF7L2* gene polymorphism.
Results
No association was found between the rs7903146 polymorphisms of *TCF7L2* gene and susceptibility to T2DM, using both dominant and recessive models.
Conclusion
In our study, we could not prove the suggested association between rs7903146(C/T) polymorphism of the *TCF7L2* gene and T2DM in Egyptians. Further Egyptian studies are needed to confirm the results of this study.

Keywords:
transcription factor 7-like 2, type 2 diabetes, SNP

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Introduction

There are 425 million people with diabetes in the world according to the International Diabetes Federation, where an estimate of eight million are present in Egypt in 2017 which is expected to rise to 16.7 million in 2045 [1]. Type 2 diabetes mellitus (T2DM) is the most common cause of diabetes representing about 90–95% of diabetic cases among the whole world. It occurs usually in individuals over 40 years old and it is characterized by hyperglycemia due to decreased insulin secretion by pancreatic beta cells, increased insulin resistance, or a combination of both [2].

T2DM is a multifactorial genetic disease, which is determined by several different genes and environmental factors [3].

More than 70 loci were identified using genome-wide association studies, which could be associated with T2DM including transcription factor 7-like 2 (*TCF7L2*) gene that is located on chromosome 10q25.3 [4]. Several potential mechanisms are present to explain the involvement of *TCF7L2* gene variants in T2DM including its effect on pancreatic islet cells development, beta-cell survival, and insulin secretory granule function [5]. Furthermore, TCF7L2 polymorphisms might cause a defect in incretin-induced stimulus secretion coupling, which mediates a reduction of insulin secretion. Such hypothesis is due to the involvement of TCF7L2, as an essential component of the wingless-type MMTV integration site family, member 1 (WnT) signaling pathway, which is crucial for the secretion of incretin hormone glucagon-like peptide-1 (GLP-1) by the intestinal endocrine L cells, leading to decreased production of postprandial insulin [6].

Among the most common studies single-nucleotide polymorphisms (SNP) in TCF7L2 which can be associated with T2DM (due to any of the previously mentioned mechanisms) is the rs7903146 (C/T) variant. It was studied in different ethnic groups which showed controversial findings [7–11].

Aim

The aim of the study was to examine the association between rs7903146 (C/T) polymorphisms of the *TCF7L2* gene, and T2DM in a sample of Egyptians.

Patients and methods

The study included 42 Egyptian patients with type 2 diabetes and 42 healthy individuals with negative family history of diabetes as a control group.

The study protocol was approved by the ethics committee of the Medical Research Institute, Alexandria University, and all contributors provided informed written consent to be included in the study.

A full medical history was taken from all participants, and a complete physical examination was performed to all of them. BMI was measured and fasting serum sample was obtained from each individual for measuring lipid profile (total cholesterol, triglycerides, high-density lipoprotein, and low-density lipoprotein-cholesterol), glucose and insulin, 2-h postprandial; another serum sample was obtained to assay postprandial blood glucose. Homeostatic model assessment for insulin resistance (HOMA-IR) was calculated using the formula: fasting glucose×fasting insulin/405 (glucose measured in mg/dl and insulin in μU/ml).

An EDTA plasma sample was collected for the molecular study of rs7903146 polymorphisms of the *TCF7L2* gene where DNA was extracted using the salting-out technique. An allele-specific PCR technique was used in the amplification (using Veriti Thermal Cycler; Applied Biosystems, Foster City, CA, USA) and the detection of the specific alleles where two sets of commercial primers were used; one for the C allele and one of the T allele of rs7903146 as follows:

(1) F-GAACAATTAGAGAGCTAAGCACTTT TTAGAAAC (forward primer specific for allele C detection).
(2) F-GAACAATTAGAGAGCTAAGCACTTT TTAGAGAT (forward primer specific for allele T detection).
(3) AGATGAAATGTAGCAGTGAAGTGC (common reverse primer).

The PCR products were analyzed by electrophoresis on 2% Tris-acetate-EDTA/ethidium bromide agarose gel, visualized under ultraviolet illumination and the presence of a 205-bp band in PCR C or T indicated the presence of the allele.

Results

Statistical analysis of data

Data were analyzed using SPSS software (version 20.0; SPSS Inc., Chicago, Illinois, USA). Qualitative data were described using number and percentage, while quantitative data were described using range (minimum and maximum), mean, SD, and median. Comparison between different groups with regard to categorical variables was made using the c^2 test. When more than 20% of the cells have an expected count of less than 5, correction for c^2 was conducted using Fisher's exact test or Monte Carlo correction. The distributions of quantitative variables were tested for normality using the Kolmogorov–Smirnov test, and if it revealed normal data distribution, parametric tests were applied (analysis of variance with the Scheffe post-hoc test). If the data were abnormally distributed, nonparametric tests were used (Kruskal–Wallis then pairwise comparison using Mann–Whitney test). Significance test results were quoted as two-tailed probabilities and the significance of the obtained results was judged at the 5% level.

The study included 42 patients with T2DM and 42 normal controls. They were recruited from the Internal Medicine Department, Medical Research Institute, Alexandria University.

Demographic, clinical, and routine biochemical serum parameters are shown in Table 1.

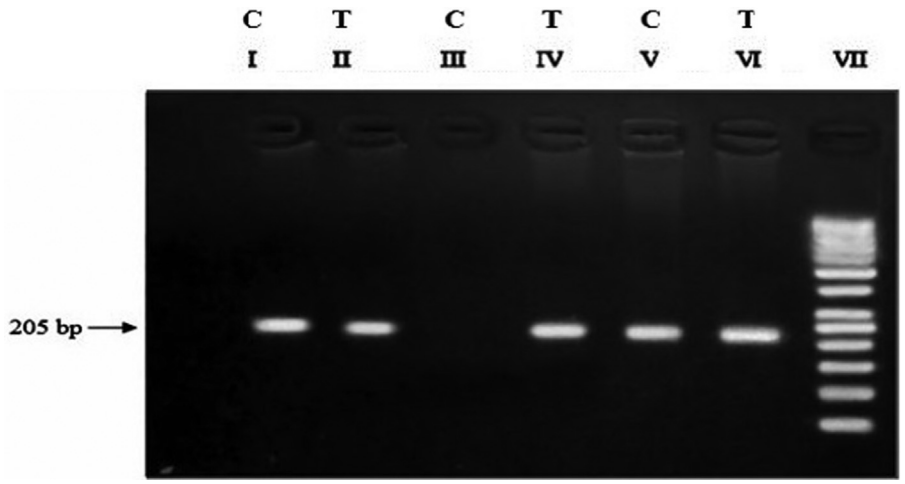
Diabetic patients had a statistically significantly higher BMI, systolic blood pressure, diastolic blood pressure, fasting blood glucose, postprandial blood glucose, HOMA-IR, triglycerides, total cholesterol, and low-

Table 1 Demographic, clinical, and routine biochemical serum parameters

Characteristics	Cases (*N*=42)	Controls (*N*=42)	*P* value
Age (years)	50.45±9.04	45.15±8.77	0.09
Sex (%)			0.12
Male	42.5	47.5	
Female	57.5	52.5	
BMI (kg/m^2)	32.43±2.0	27.61±2.27	0.001*
SBP (mmHg)	144±19.1	112±12.0	0.001*
DBP (mmHg)	93.0±12.2	74.4±8.60	0.001*
FBG (mg/dl)	208.67±47.46	90.02±11.39	0.001*
PPG (mg/dl)	278.58±52.02	93.87±11.59	0.001*
TCH (mg/dl)	218.85±32.36	185.60±26.70	0.001*
LDL (mg/dl)	132.28±35.48	96.46±30.11	0.001*
HDL (mg/dl)	59.50±11.40	68.93±8.27	0.001*
TG (mg/dl)	134.0±43.98	101.10±27.30	0.001*
Insulin(μIU/ml)	14.21±12.69	9.9±10.66	0.066
HOMA-IR	6.97±5.43	2.1±2.23	0.001*

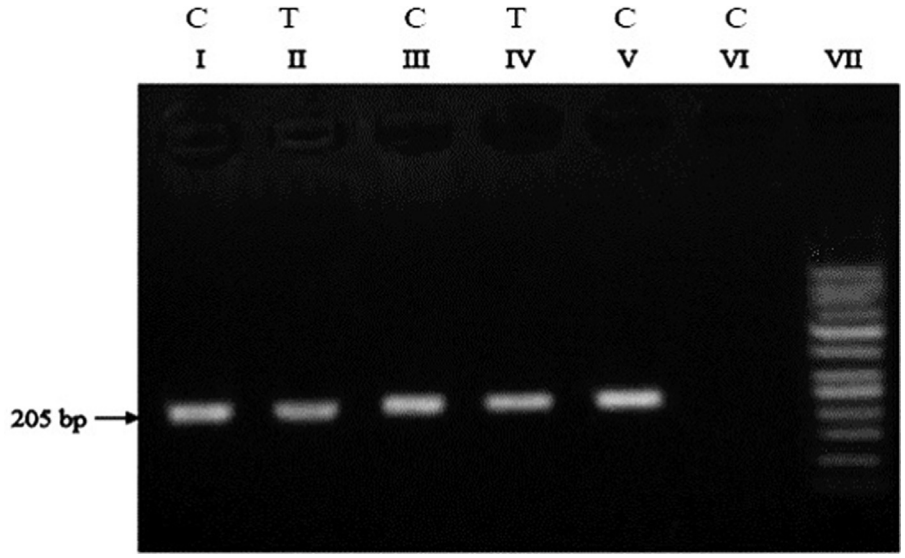
DBP, diastolic blood pressure; FBG, fasting blood glucose; HDL, high-density lipoprotein cholesterol; HOMA-IR, homeostatic model assessment for insulin resistance; LDL, low-density lipoprotein; PPG, postprandial blood glucose; SBP, systolic blood pressure; TCH, total cholesterol; TG, triglycerides. *Statistically significant at *P* value less than or equal to 0.05.

Figure 1



Allele-specific PCR genotyping of rs7903146C/T. Lanes I and II: PCR product of the same patient showing amplification of alleles C and T (band at 205 bp) – heterozygous genotype CT. Lanes III and IV: PCR product of the same patient showing absence of amplification of allele C at lane III and amplification of allele T at lane IV, respectively (band at 205 bp) – homozygous genotype TT. Lanes V and VI: PCR product of the same patient showing amplification of alleles C and T, respectively (band at 205 bp) – heterozygous genotype CT. Lane VII: molecular weight marker (50 bp DNA ladder).

Figure 2



Allele-specific PCR genotyping of rs7903146C/T. Lanes I and II: PCR product of the same patient showing amplification of alleles C and T, respectively (band at 205 bp) –heterozygous genotype CT. Lanes III and IV: PCR product of the same patient showing amplification of alleles C and T, respectively (band at 205 bp) – heterozygous genotype CT. Lanes V and VI: PCR product of the same patient showing amplification of allele C at lane V (band at 205 bp) and absence of amplification of allele T at lane VI – homozygous genotype CC. Lane VII: molecular weight marker (50 bp DNA ladder).

density lipoprotein, but significantly lower high-density lipoprotein compared with controls. Insulin level was higher in patients than in controls but the difference was not significant.

Molecular genetic results

Allele-specific PCR was used to genotype rs7903146(C/T) in both cases and controls. The presence of the T allele was confirmed by detecting a 205 bp product as shown in Figs 1 and 2. The observed TCF7L2 rs7903146 genotype frequencies in this study were in Hardy–Weinberg equilibrium as shown in Table 2. Detected by the allele-specific PCR technique, as shown in Table 3, the frequency of CC, CT, and TT genotypes in the patients' group were 16.7, 61.9, and 21.4%, respectively. The frequency of CC, CT, and TT genotypes in controls were 11.9, 57.1, and 31%, respectively. The T-allele frequency in patients was 52.4% while the C-allele frequency was 47.6%. The T-allele frequency in controls was 59.9% while the C-allele frequency was

found to be 40.5% (Table 3). It was noticed that the TT genotypes and the T-allele frequency was higher in controls than cases. The presence of the T allele was confirmed by detecting a 205 bp product as shown in Figs 1 and 2. Column charts (Figs 3 and 4) show a comparison of genotype and allele distribution between case and control groups, respectively.

On the basis of the c^2 test, the result was nonsignificant regarding comparison between different genotypes within the case group or within the control group regarding the relation between genotype and susceptibility to T2DM (Table 3). Furthermore, using dominant and recessive models, the test result was nonsignificant regarding the relation between genotype and susceptibility to T2DM (Table 4).

Statistical testing was done to explore the impact of the rs7903146 (C/T) polymorphism on biological characteristics including the age of onset, BMI, lipid profile, FBS, postprandial blood glucose, fasting insulin level, and HOMA-IR; however, no statistical association could be detected between the studied polymorphism and any of these parameters.

Table 2 Observed and expected values of genotype frequencies among the two groups and total sample

Genotype	Observed	Expected	χ^2	*P*
Group I				
CC	7.0	9.5	2.437	0.118
CT	26.0	21.0		
TT	9.0	11.5		
Group II				
CC	5	6.9	1.451	0.228
CT	24	20.2		
TT	13	14.9		
Total sample				
CC	12.0	16.3	3.619	0.057
CT	50.0	41.4		
TT	22.0	26.3		

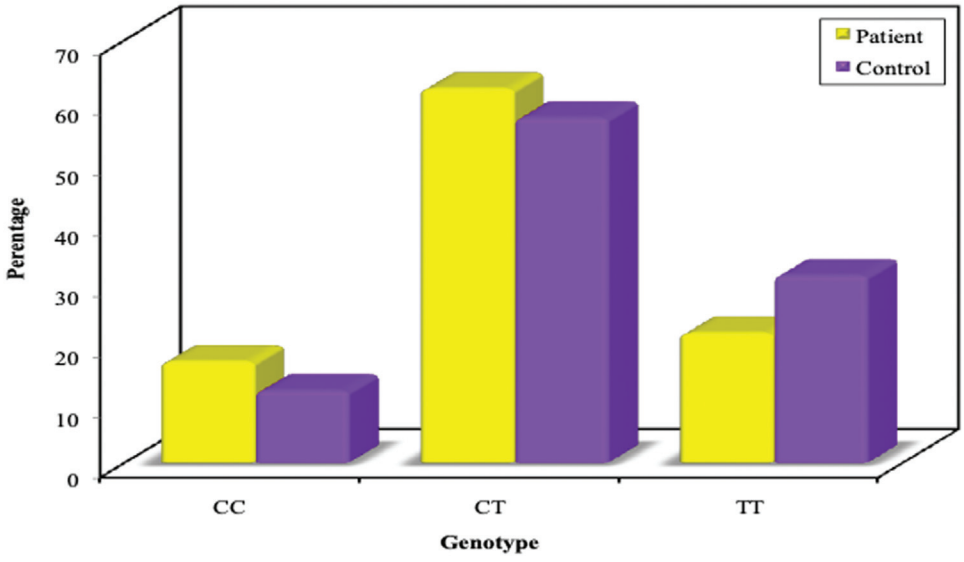
Discussion

T2DM is a very common metabolic disorder of polygenic nature, characterized by impaired insulin secretion, peripheral insulin resistance, and increased hepatic glucose output leading to hyperglycemia [12]. Genome-wide association studies have identified several novel susceptibility genes, with varied T2DM susceptibility across several ethnic groups. One of these genes is *TCF7L2* gene [13].

TCF7L2 forms heterodimers with ß-catenin, inducing the expression of various genes, including the insulinotropic hormone GLP-1 gene [6], the insulin gene [14], and other genes that encode proteins involved in processing and exocytosis of insulin granules [15]. Incretin-based therapy is ideal for T2DM management because of its efficacy, good tolerability, low risk of hypoglycemia, and weight loss [16]. This class of treatment includes GLP-1 receptor agonists and dipeptidyl peptidase-4 inhibitors. GLP-1 receptor agonists have been proved to reduce hemoglobin A1c levels by 0.8–1.5% [17].

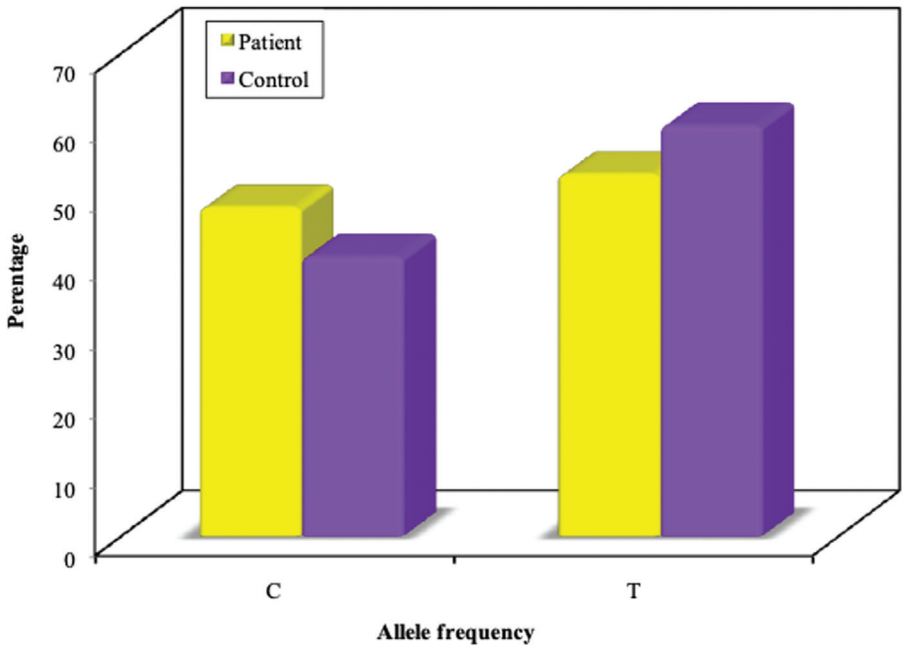
SNPs of the *TCF7L2* gene have been reported to affect T2DM susceptibility by indirectly altering the

Figure 3



Comparison between the studied groups according to genotype.

Figure 4



Comparison between the studied groups according to allele frequency.

Table 3 Comparison between the studied groups according to genotype

	Patient (*N*=42) [*n* (%)]	Control (*N*=42) [*n* (%)]	χ^2	*P*	OR	95% CI (LL–UL)
Genotype						
CC	7 (16.7)	5 (11.9)	1.141	0.565	1.480	0.429–5.100
CT	26 (61.9)	24 (57.1)			1.219	0.509–2.916
TT	9 (21.4)	13 (31.0)			0.608	0.227–1.630
Allele frequency						
C	40 (47.6)	34 (40.5)	0.869	0.351	1.337	0.726–2.463
T	44 (52.4)	50 (59.5)			0.748	0.406–1.378

χ^2, χ^2 test; CI, confidence interval; OR, odds ratio.

Table 4 Comparison between the studied groups according to genotype using dominant and recessive models

	Patient (*N*=42) [*n* (%)]	Control (*N*=42) [*n* (%)]	χ^2	*P*	OR	95% CI (LL–UL)
Genotype						
RecessiveCC+CT	33 (78.6)	29 (69.0)	0.985	0.321	1.644	0.614–4.404
TT	9 (21.4)	13 (31.0)			0.608	0.227–1.630
Dominant						
?CC	7 (16.7)	5 (11.9)	0.389	0.533	1.480	0.429–5.100
TT+CT	35 (83.3)	37 (88.1)			0.676	0.196–2.328

CI, confidence interval; OR, odds ratio.

expression of GLP-1 [18], which in addition to insulin plays a critical role in blood glucose homeostasis [6]. Some clinical data have suggested that polymorphism affected the capacity of pancreatic ß-cells to secrete insulin rather than aggravating insulin resistance [7,8].

The aim of the present study was to investigate the association of rs7903146(C/T) polymorphism of the *TCF7L2* gene with T2DM.

Although disease association studies have shown that the T allele of the rs7903146 SNP at the TCF7L2 locus is strongly associated with T2DM risk in diverse ethnic groups, it is noteworthy that the frequency of SNPs at this locus was shown to be different in the various populations included in these studies [9,13,19].

The T allele of rs7903146 SNP at the TCF7L2 was seen in 18–42% of nondiabetic European and Arab populations [19–21], but in less than 5% of healthy

individuals in studies with Southeast Asian populations [22,23].

Several studies have reported the same findings. In the meta-analysis of Cauchi *et al.* [13], they investigated the association between the TCF7L2 rs7903146 polymorphism and T2DM in Moroccans. They found a reproducible association with T2DM. In Tunisia, Turki *et al.* [24] found a strong contribution of *TCF7L2* gene variants to T2DM among Tunisians confirming TCF7L2 as a common T2DM candidate gene. In addition, in Ghana, a study investigated the association between the rs7903146 polymorphism and T2DM in the Ghanaian population. The study showed an association between rs7903146 polymorphism and T2DM in Ghanaian individuals [25].In Palestine, a strong association between rs7903146 and T2DM was found [26]. In India, Sanghera *et al.* [27] found an association of rs7903146 polymorphism with T2DM in Indian Sikh. Also in Iran, in a study by Amoli *et al.* [28] they found an association between the rs7903146 T allele and T2DM in an Iranian population.

The results of the present study, in contradiction with the previous studies, found no association between rs7903146 and T2DM. In agreement with our findings, Wang *et al.* [9] found that the rs7903146 variant has no association with T2DM in Han Chinese people.

In Saudi Arabia, Alsmadi *et al.* [11] found weak or no association of T2DM in Arabs with the *TCF7L2* rs7903146 variant. The T-allele frequency of the diabetic patients was not different from that observed in the controls.

In Thailand, Tangjittipokin *et al.* [29] did not find an association between rs7903146 and T2DM in Thai population. The variability seen in the association results may be due to many factors such as ethnic stratification, sample size, or gene–gene and gene–environment interactions.

This study found no association between rs7903146(C/T) and T2DM. Further investigation of this gene using a larger sample size and conducting more molecular studies to reveal more of the genes involved in the pathogenesis of T2DM is important to explore more options for the treatment of patients and high-risk carriers and for a more proper drug selection.

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Nil.

Conflicts of interest
There are no conflicts of interest.

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