The Relationship Between rs7903146C>T in TCF7L2 Gene and Type 2 Diabetes mellitus in Iraq Populations

Assist.Prof.Dr. Rasha Shakir Nima Al-Aridhi Assist.Prof. Dr.Rasha Amer Noori Al-TufailiUniversity of Kufa/Faculty of Science

rasha.alaridhi@uokufa.edu.iq rashaa.altufaili@uokufa.edu.iq

Abstract:

Background: Transcriptional factor 7-like 2 (TCF7L2) is a transcription factor in the Wnt-signaling pathway, expressed in many tissues including fat, liver and pancreatic islets of Langerhans. The relationship between TCF7L2 and type 2 diabetic mellitus (T2D) has been determined in several studies, but the data are dubious. *Objective:* the sole aim of this study is to determine the relationship between rs7903146C>T SNP in TCF7L2 gene with T2D in the Iraqi population. *Methods:* Two groups of Iraqi patients were recruited: 210 Type 2 Diabetic patients without complication and 210 aged-matched healthy subjects as the control group. The rs7903146C>T in TCF7L2 gene was detected using polymerase chain reaction-ARMS method. *Results:* The experimental results pointed out significant differences in the body mass index, west circumference, fasting blood glucose levels, HbA1c, triacylglycerol, very low density lipoprotein, high density lipoprotein cholesterol, total cholesterol, insulin, and HOMA-IR in T2D group with respect to control group. Interestingly we observed the frequency of homozygote mutant TT, heterozygote TC, and homozygote wild CC genotype of rs7903146C>T polymorphism in T2D group were 55.07%, 33.33%, and 11.59% respectively. Additionally, we identified a significant association between the SNP, haplotypes of HbA1C, fasting glucose, fasting insulin and HOMA-IR. We conclude that the variant rs7903146C>T in TCF7L2 gene is associated with T2D. Thus, this genetic test can be used to predict the occurrence of T2D in Iraqi population.

key words :TCF7L2 Gene and Type 2 Diabetes mellitus

Introduction:

Presently, it has informed that about 381.8 million subjects grieved from diabetes mellitus in 2013 around the world. This number will develop by 55% in 2035, so

about 591.9 million subjects will be affected with Diabetes mellitus (Cannon *et al*. 2018)

One of the most types of diabetes is T2D. It is characterized by disturbances in carbohydrates and chronic hyperglycemia. It has confirmed that T2D results from the impaired of insulin secretion, hepatic glucose output increase, and insulin resistance in peripheral tissues (Guillausseau *et al.* 2008). Both genetic and environmental factors work together and cause T2D. Several susceptibility genes associated with T2D, acknowledged by the high output GWA studies (Sladek *et al.* 2007 & Kwon *et al.* 2018).

Numerous studies have been conducted on TCF7L2 gene polymorphisms and its correlation with T2D. A transcription factor in the Wnt-signaling pathway is transcriptional factor 7-like 2 (TCF7L2), expressed in many tissues including fat, liver and pancreatic islets of Langerhans (Shokouhi *et al.* 2014). Shu *et al*. had displayed that TCF7L2 expression in the β-cells is correlated with the function and captured of the insulin forming pancreatic β-cell (Shu *et al.* 2009). The well-determined variants of TCF7L2 gene having clinical significance are (rs7903146 C/T) of intron 3, (rs791695 T/C) of intron 3, (rs12255372 G/T) of intron4 and (rs11196205 G/C) of intron 4 (Tong *et al.* 2009).

The relationship between TCF7L2 and T2D has been determined in several studies, but the data are dubious (Maller *et al.* 2012). Shokouhi *et al*. have exhibited that rs1494556, rs199473684, and rs290484 SNPs in TCF7L2 gene are associated with T2D in kurdish ethnic group of Iranian population (Shokouhi *et al.* 2014). The variants of TCF7L2 gene have been investigated in Chinese people. The results didn't exhibit significant difference (Chang *et al.* 2009). The rs7903146 SNP in TCF7L2 is greatly determined to be creative via analysis in different ethnicities (Palmer *et al.* 2011 and Helgason *et al.* 2007). It has been found the T allele of rs7903146 is a risk for the development of T2D in European and African individuals, in contrast to the Han Chinese individuals. This may define the conflicting data among analysis in various populations (Basile *et al.* 2014). So, this study was strived to study the association rs7903146C>T SNP in TCF7L2 gene with Type 2 diabetics in the Iraqi population.

Materials and Methods:

Patient & Control Groups:

A case-control study was conducted on 420 Iraqi subjects. The age of patients was >40 years old, who attended the diabetic center at Al-Sadder Medical City, Najaf, Iraq. Two hundred and ten type2 diabetic patients without complications, and 210 healthy control. Patients were diagnosed by specialized physicians with the use of the appropriate criteria of diagnosis of diabetes mellitus. The study protocol was approved by the Ethics Committee of the College of Science at the University of Kufa. Written informed consent was obtained from the patients. Overnight fasting venous blood samples from all subjects were collected from 8:00 to 9:00 am, weight, height, and west circumference were measured in standing position without shoes. The blood specimens were separated into two parts, the first was separated into serum for biochemical assays, and the second was collected in tubes containing EDTA as anticoagulant agent for HbA1C using Glycosylated Hemoglobin (GHb/HbA1c) Colorimetric Assay Kit, Elabscience, catalog number E-BC-k089 , and for genomic DNA extraction by using Promega kit , cat number A1120.

The sera were used for biochemical assays. Total cholesterol, Triglyceride, high density lipoprotein-cholesterol, low density lipoprotein-cholesterol, very low density lipoprotein, and fasting blood glucose were measured by an automatic biochemical analyzer.

Genotyping of Transcriptional Factor 7-Like 2 SNP(rs7903146 C>T) A rapid and simple allele-specific PCR method was used for genotyping of rs7903146C>T SNP in TCF7L2 gene. Two forward primers with a mismatch in their last 3′ nucleotide were included, such a way that each is specific for one of the two variants of the polymorphism (Forward C ;GAACAATTAGAGAGCTAAGCACTTTTTAGAAAC. Forward T; GAACAATTAGAGAGCTAAGCACTTTTTAGAGAT. Common reverse; AGATGAAATGTAGCAGTGAAGTGC) (Dutra *et al.* 2008). For each sample, two PCR reactions were run in parallel, one with 1µl (10pmole) primers rs7903146 C and 1µl (10pmole) rs7903146 R, and the second with primers, 1µl (10pmole) for rs7903146 T and 1µl(10pmole) for rs7903146 R (PCR T), each, 5µl Accpower PCR PreMix. In addition to 100 ng/µl template DNA and completed the volume to 20µl with nuclease free water. The PCR products were analyzed by

electrophoresis on 2% Tris–acetate-EDTA/ethidium bromide agarose gels, visualized under ultra-violet illumination and the presence of the 205 bp-band (C or T) indicated the presence of the allele(as in figure 1). A sample was considered negative for a particular allele when the amplicon was absent. Furthermore, as the DNA starting material for each allele-specific genotyping reaction came for the same DNA sample, one control the other in detecting either a false negative reaction secondary to extraction failure or the presence of an inhibitor.

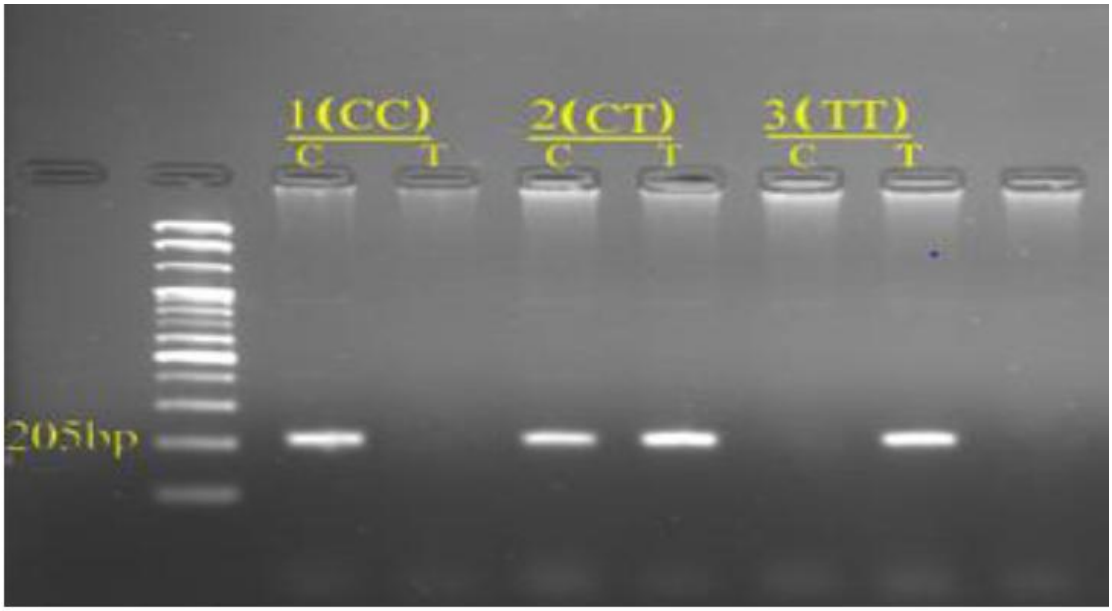

Figure1: Results of genotyping of rs7903146C>T in Transcriptional Factor 7-like 2 by allele–specific PCR.

Statistical analysis:

The Results are presented as mean ± standard deviation. Statistical analysis was made by SPSS (SPSS Inc., Chicago, IL, USA). Chi secure tests were performed to consider the deviation from Hardy –Weinberg equilibrium.

The Results:

The characteristic results of T2D and control group results are summarized in table 1. There were significant differences in body mass index and waist circumference between the two groups. However, no significant difference in age and gender (female/male ratio).

Table 1: Characteristics of type 2 diabetic and control group.

Parameter	Control N=210	T2D N=210	P (value)
	Mean± SD	Mean± SD	
Age (year)	59.9±8.60	57.3±9.8	**N.S**
Female/male	108/102	110/100	**N.S**
BMI(Kg/m^2)	25.775±2.97	29.450±6.8	**S**
WC (cm)	89.7±17.92	99.5±22.73	**S**

BMI; body mass index, and WC: Waist Circumference

All the clinical data were shown in table 2. Both groups significantly differ in cardiovascular risk factors (very low density lipoprotein, triglyceride, high density lipoprotein –cholesterol, low density lipoprotein- cholesterol, and total cholesterol). As expected, the group with T2D showed higher levels of glycosylated hemoglobin, fasting blood glucose, insulin level, and insulin resistance.

Table2: Clinical data of subject with diabetic and healthy controls.

Parameter	Control N=210	T2D N=210	P (value)
	Mean± SD	Mean± SD	
FBG (mg/dl)	80.3±9.56	210.1±99.65	S
HbA1c (%)	5.95±0.506	9.01±1.971	S
VLDL (mg/dl)	12.549±6.969	35.495±20.761	S
TG (mg/dl)	105.736±18.736	238.339±135.09	S
HDLc (mg/dl)	59.045±6.766	41.056±10.961	S
LDLc (mg/dl)	83.103±18.119	145.160±64.486	S
TC (mg/dl)	130.413±36.489	215.352±64.576	S
Insulin (µU/ml)	10.295±3.509	27.507±19.85	S
HOMA-IR	1.36±0.484	13.3±10.881	S

FBG: Fasting Blood Glucose, VLDL: Very low density lipoprotein, TG: Triglycerides, HDLc: high density lipoprotein cholesterol, LDLc: Low density lipoprotein cholesterol, TC: Total cholesterol, HOMA-IR:

The genotyping and allele frequency distribution in the two studied groups and the deviation from HWE are reported in table 3. The genotyping frequencies of the selected SNP are within HWE in T2D and control group, suggesting that there is no systematic problem with rs7903146C>T. The frequencies of studied SNP in TCF7L2 gene were the highlight to be 55.07% for the homozygote TT, 33.33% for heterozygote TC, and 11.59% for the homozygote CC in T2D group. The CC and TT allele frequencies were observed to be 28.261% and 71.739% respectively in T2D.

Also, the analysis of rs7903146C>T in Control group revealed the frequencies of 61.29%, 32.45%, and 6.45% for CC, CT, and TT genotypes respectively. And the minor alleles frequencies of C and T types were observed to be 77.419 and 22.581 respectively.

Table 3: Genetic analysis of rs7903146C>T SNP in TCF7L2 gene and deviation from Hardy –Weinberg equilibrium (HWE) in control and T2D.

	Genotyping number/ frequency (%)			P Value
	CC	CT	TT	
Control (210)	129/61.29	68/32.45	13/6.45	**N.S**
T2D (210)	24/11.59	70/33.334	116/55.23	**N.S**
	Allelic frequency			
	C	T		
Controls	77.419	22.581		
T2D	28.261	71.739		

The genetic models results in table 4, showed that there was evidence of a genetic Co-dominant, dominant, and recessive models for rs7903146 C>T in TCF7L2 gene differed significantly between the two groups. The results demonstrated that TCF7L2 rs7903146C>T was significantly associated with T2D for alleles, and for genotypes. The minor allele was T and the major allele was C. The homozygotes TT had about one the T2D risk of allele C carriers (OR 1) under various inheritance genetic models.

Table 4: Genotyping of rs7903146C>T in TCF7L2 gene in T2D and control groups

Model	Genotype	Numbers		OR (95%CI)	P Value
		T2D	Control		
Co dominant	CC	24	129	1.00	
	CT	70	68	5.463(2.071-14.406)	S
	TT	116	13	45.125(12.525-162.576	
Dominant	CC	24	129	1.00	
	CT+TT	186	81	12.0729(4.924-29.601)	S

Recessive	CC+CT	94	197	1.00	S
	TT	116	13	17.7742(5.807-54.409)	
Over dominant	CC+TT	140	142	1.00	N.S
	CT	70	68	1.050(0.506-2.1806)	

The biochemical characteristics of patients with T2D were analyzed in relevance to the genotype of the studied SNP in TCF7L2 gene under the dominant model. There was a significant association between genotype (CC vs. CT+TT) and plasma HbA1c, Fasting Glucose, Fasting glucose level and insulin resistance (table 5).

Table 5: Difference in the gene variations and related biochemical characteristic in T2D Patients

Biochemical Parameters	**CC**	**CT+TT**	**P Value**
HbA1C %	8.321 ± 1.025	10.202 ± 2.422	**S**
Fasting glucose (mg/dl)	151.57 ± 52.21	242.93 ± 64.92	**S**
Fasting insulin µU/ml	38.538±20.365	20.365 ± 2.972	**S**
HOMA-IR	18.167±8.433	10.351 ± 9.421	**S**

The Discussion:

Type 2 diabetes mellitus is really serious global health problem. Around the world, T2D comprises the majority of people with diabetes. In 2016 WHO evaluated that diabetes was the seventh leading cause of death. Until recently, T2D was gotten only in adults but it is now also occurring increasingly frequently in children (Global report of diabetes, 2018).

Type 2 diabetes mellitus is determined by several different genes and environmental factors. Several previous researches have been carried out to evaluate the genetic factors impacted in T2D. New technologies, such as the discovery of SNPs will lead to a better understanding of the pathogenesis of T2D and to better diagnostics,

treatment, and ultimately avoidance (Tilburg *et al*. 2001). Disturbance of glucose metabolism; elevated levels of gastric inhibitory peptide and glycated hemoglobin (HbA1c) and decreases processing of proinsulin, can be observed in normoglycemic individuals with TCF7L2 polymorphisms before the onset of type 2 diabetes (Gjesing *et al.* 2011 & Gautier *et al.* 2011). Thus, the polymorphisms in TCF7L2 gene are thought to have effects on pancreatic β cells that impair insulin secretion (Chiang *et al.* 2012).

The mechanisms underlying the association between the rs7903146C>T and T2D are not yet well understood. The mutation rs7903146C>T in TCF7L2 reside in an intronic region. So it is reasonable to suppose a regulatory process is implicated, in advising T2D risk (Xia *et al.* 2014). The previous study found that this variant within the TCF7L2 gene belongs to an element that controls the expression of acyl-CoA synthases 5 (ACsL5). Also, it should be noted that ACsL5 play an important role in fatty acid degradation and lipid synthesis, Which could be correlated with T2D (Boman *et al.* 2016). Furthermore, the earlier study indicated that beta-catenin (multitasking and evolutionarily conserved molecule) plays a serious role in modifying insulin secretion, moreover that the overexpression of the transcriptional co-activator of beta-catenin, TCF7L mitigates insulin secretion (Sorrenson et al. 2016). Another hypothesis induced that the minor allele T of rs7903146C>T is a significant risk factor for damaged pro-insulin conversion (Shen *et al.* 2015). Besides, there are still other likely mechanisms by which TCF7L2 gene polymorphisms may be involved in the initiation of T2D (Oh *et al.* 2017) that remain to discovered.

Previous studies found that the minor T allele frequency in rs7903146 C>T in TCF7L2 gene was 36.15% in the Arab population (Alsmadi *et al.* 2008) 34.45% in the Iranian population (Alami *et al.* 2012) and 30.15% in the Czech population (Vcelak *et al.* 2012). So the results of the current study consistent with the previous researches in varied ethnic populations. The results of the present study pointed to the fact that rs7903146 C>T in TCF7L2 gene is a risk of T2D in the Iraqi population. Currently, rs7903146 C>T in TCF7L2 gene can be considered as important tools to identify the Iraqi population at risk.

Conclusion: We conclude that the variant rs7903146C>T in TCF7L2 gene, is associated with T2D in the Iraqi population. Additionally, might play an important role in explaining these traits and to understand the biological and genetic mechanisms underlying T2D.study are consisted with the previous researches in varied ethic populations. The results of the present study pointed to the fact that rs7903146 C>T in TCF7L2 gene is a risk of T2D in Iraqi population. Currently, rs7903146 C>T in TCF7L2 gene can be considered as important tools to identify the Iraqi population at risk.

Conclusion: We conclude that the variant rs7903146C>T in TCF7L2, is associated with T2D in Iraqi population. Additionally, might play an important role in explaining these traits and to understand the biological and genetic mechanisms underlying T2D.

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