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Article

Transcription Factor 7- Like -2 (TCF7L2) rs7903146 (C/T) polymorphism in Iraqi patients with Type 2 Diabetes Mellitus

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ABSTRACT

Type 2 diabetes (T2D) is a metabolic disorder that develops as different cell groups resist insulin action on peripheral tissues. Eventually, the pancreas cannot produce sufficient insulin to overcome this resistance, resulting in insulin deficiency. The transcription factor 7-like-2 gene (TCF7L2) rs7903146 (C/T) polymorphism is one of the most susceptible genes to T2DM discovered to date, with the contribution to the disease through the Wnt/β-catenin signaling pathway affecting pancreatic islet development. To investigate and analyze the correlation of TCF7L2 gene polymorphisms and their association with type 2 diabetes for Iraqi patients. This study included 80 blood samples equally divided into two groups: patients with T2DM and normal healthy controls. All Genotypes of rs7903146 (C/T) SNP in theTCF7L2 gene were evaluated by real-time polymerase chain reaction via TaqMan allelic discrimination. Analysis of the distribution of the TCF7L2 rs7903146 genotype and allele revealed that the TT genotype was more frequent in the T2DM group (32.5%) than in healthy controls (12.5%) **(**OR = 5.9, 95% confidence interval (CI = 1.6–20.6, p = 0.05). The T allele was more frequent in diabetic patients (52.5%) than healthy control (25%), and it was associated with high risk of diabetes (odd ratio = 3.3, 95% CI= 1.6- 6.4), P=0.0005. **Conclusion:** The T allele of rs7903146 polymorphism of TCF7L2 confers susceptibility to the development of T2DM in the Iraqi population.

Keywords: Type 2 diabetes mellitus · Transcription factor 7-like-2 ·rs7903146 polymorphism

INTRODUCTION

Diabetes is a disease that is defined by a chronic state of hyperglycemia, and that occurs when the pancreas does not produce enough insulin or when the body cannot effectively use the insulin it produces; uncontrolled diabetes causes hyperglycemia, or an increase in blood sugar, which over time causes significant damage to the body's systems, particularly the neurons and blood vessels[1]

TypeType 2diabetes mellitus (T2DM) represents a group of polygenic metabolic and endocrine disorders with various genetics and environmental influences that affect the capacity of the body to produce or use insulin, resulting in hyperglycemia, which may lead to variable complication[2] It has become one of

the foremost chronic non-communicable diseases distressing the health of people worldwid[3].

The global prevalence is increasing at a dreadful rate, making it the most dreaded silent epidemic of the twenty-first century. According to the International Diabetes Federation, 451 million people had diabetes in 2017, projected to reach 693 million by 2045[4]. There were 1.411.500 cases of diabetes in Iraq in 2017; the total adult population[5]

The development of research and studies have led to the discovery of many genes that are related to the development of type 2 diabetes; from these genes, The transcription factor 7-like 2 genes (TCF7L2) which has a significant relationship with diabetes[6,7] And there is emerging evidence that some genetic polymorphisms can impact the risk of evolving T2DM [2]

The TCF7L2 gene spans around 215,863 bases on the chromosome 10q25.3. The TCF7L2 codes for a transcription factor tangled in the Wnt signaling pathway, which plays an important role in adipogenesis and development of pancreatic islets. Also, TCF7L2 plays a significant role in controlling the biosynthesis, processing and secretion of insulin[8]

To investigate and analyze the correlation of TCF7L2 gene polymorphisms and their association with type 2 diabetes for Iraqi patients.

MATERIALS AND METHODS

Subjects:-This study was carried out in the Institute of Genetic Engineering and Biotechnology for Postgraduate Studies laboratories - University of Baghdad and Iraqi Hereditary Company in Al-Harthiya from 1 November 2021 until the end of Julie 2022. This study included 80 blood samples equally divided into two groups: patients with T2DM and normal healthy controls. Blood samples were collected from both groups for biochemical examinations and molecular study. The age of patients and normal healthy controls ranged from 25 to 70 years. Age, BMI, fasting blood glucose, glycated hemoglobin and lipid profiles were evaluated in serum specimens of T2DM patients and normal healthy controls. Exclusion Criteria of T2DM were: 1. any subjects diagnosed with T1DM who are on insulin therapy. 2. Pregnant women, patients with renal disease, liver disease, thyroid disorders or other endocrine or chronic diseases and Alcoholism.

Every participant has been interviewed and asked to answer information including age, family history, weight, height, and disease duration.

Biochemical Tests Assay

Biochemical tests involving fasting blood sugar, HbA1C and lipid profile test include total Cholesterol tests, Triglyceride tests, HDL tests, VLDL tests and LDL tests. Kits that are used in this method are from AGAPPE Company / Switzerland.

Genomic DNA Extraction and Genotyping:

The DNA was isolated from the EDTA blood sample by using a DNA extraction kit (Addprep / Korea) following the manufacturer's procedure. The rs7903146 (C/T) SNP of TCF7L2 was genotyped using real-time PCR with TaqMan allelic discrimination. The primers and probes sequence are shown in Table 1. The probes were labeled using VIC and FAM fluorescent dyes to distinguish between the two alleles (Figure 1). Real-time PCR reactions were performed in a total volume of 20 µl including 10 µl TaqMan Genotyping Master Mix, 0.75 µl Custom TaqMan assay (primer/probe), 3 µl genomic DNA and 4 µl Nuclease free

water. The conditions for cycling were: initial denaturation at 94 °C for 5 min, followed by 35 cycles of denaturation at 94 °C for 15 s, annealing at 56 °C for 15s and extension at 72 °C for 20 s.The genotypes were classified as homozygote allele (CC), heterozygote (CT), and homozygote allele (TT).

SNP	**TCF7L2 rs7903146**		
Primer	**Sequence**	**length**	**Tm**
Forward	GCCTCAAAACCTAGCACAGC	20	62
Reverse	GCCTTCCCTGTAACTGTGGT	20	62
Probe	**Sequence**		
FAM	GATACTATATAATTTAATTGCCGTATGAGG	30	78
VIC	ATAGTATATAATTTAATTGCCCGTATGAGG	29	74

Table 1. TaqMan SNP genotyping assay information.

Statistical analysis

The Statistical Analysis System- SAS (2018) program was used to detect the effect of different factors on study parameters. T-test and Least significant difference –LSD test (Analysis of Variation-ANOVA) was used to compare between means in this study significantly. In addition, the Chi-square test was performed (0.05 and 0.01 probability) to make a meaningful comparison between percentages. This study estimated the correlation coefficient between variables (Cary, 2012). The odds ratio (OR) with a 95% confidence interval (CI) was calculated for the associated risk of T2DM.

RESULT

The detection of the genotype of TCF7L2C/T (rs7903146) gene polymorphism with allele frequencies between the study groups (patients and control) was applied using the RT-PCR technique. The genotypic frequencies of T2DM were 27.5% (n=11) normal CC and 40% (n=16) heterozygous CT. Mutant homozygous was found in TT 32.5% (n=13). In controls, the results demonstrate 62.5% (n=25) wild-type CC, 25 % (n=10) heterozygous CT and mutant homozygous TT 12.5 % (n=5). The results of genotype frequencies of T2DM analysis shown in Table (2) reveal that the wild-type genotype and wild-type allele were taken as reference. In TCF7L2 rs7903146 Polymorphism, the odd ratio for the TT genotype was 5.9(1.6-20.6) with p=0.005, indicating that homomutant genotype TT was a higher risk of T2DM than the wild type CC. The T allele was more frequent in diabetic patients (52.5%) than healthy control (25%), and it was associated with high risk of diabetes (odd ratio = 3.3, 95% CI= 1.6- 6.4), P=0.0005.

TCF7L2 polymorphism rs7903146 C >T	Frequencies (%)		P value	Odd ratio (95% CI)
	Control group (n=40)	Patients Group (n=40)		
Codominant				
CC	62.5% (n=25)	27.5% (n=11)	**---**	**1.00 (Reference)**
CT	25% (n=10)	40% (n=16)	**0.017**	**3.6 (1.2-10.5)**
TT	12.5% (n=5)	32.5% (n=13)	**0.005**	**5.9 (1.6-20.6)**
Dominant				
CC	62.5% (n=25)	27.5% (n=11)		**1.00 (Reference)**
CT+ TT	37.5% (n=15)	72.5% (n=29)	**0.0021**	**4.3 (1.7-11.2)**
Recessive				
CC+CT	87.5% (n=35)	67.5% (n=27)		**1.00 (Reference)**
TT	12.5% (n=5)	32.5% (n=13)	**0.03**	**3.3 (1.0-10.6)**
C	75% (n=60)	47.5% (n=38)		**1.00 (Reference)**
T	25% (n=20)	52.5% (n=42)	**0.0005**	**3.3 (1.6-6.4)**

Table 2. Comparison of the Genotype and Allele Frequencies of TCF7L2 gene rs7903146 polymorphism between patients with T2DM and Healthy groups.

DISCUSSION

T2DM is the most common form of DM, accounting for ~90% of cases. It has a strong genetic component amplified by several environmental and lifestyle factors.T2DM is characterized by impaired insulin action in target tissues such as muscle, liver, and fat, insulin resistance, coupled with insufficient secretion of insulin from the β-cells of the pancreatic islets. Hyperglycemia results when insulin secretion is unable to compensate for insulin resistance [9]. The TCF7L2 gene product is an essential transcription factor in the Wnt/β-catenin signaling pathway, acting on cell proliferation, polarization, embryogenesis, and tissue homeostasis. The Wnt pathway, in turn, regulates pancreatic β-cells proliferation and acts on insulin secretion. It has been discovered that changes in this pathway can alter the insulin action and resistance, facilitating the T2DM onset [10].

These results agree with Iranian Kurdish results that were studied by [11] who reported risk T allele of the rs9703146 polymorphism associated with T2DM.

The CT genotype (OR = 1.98, p =0.008), TT genotype (OR = 3.54, p = 0.024), and the dominant model (OR = 2.16, p =0.002). In Han Chinese population, it was reported that TCF7L2 (rs7903146) polymorphism is associated with T2DM. (OR=1.36, 1.24–1.48; p= 6.404610212) [11]. Furthermore, agree with [12], who found a statistically significant interaction of the rs7903147 (T) allele associated with the risk of developing T2DM. (OR=4.08, 95% CI =1.95 – 11.80) p=0.0001). In Egypt, [13] found the T allele of the rs7903146(C/T) SNP was associated with a high risk of development of T2DM with an OR of 1.35 (95% CI: 0.68–2.6) and the heterozygous genotype (CT) with an OR 1.16 (95% CI: 0.49–2.7); however, they were statistically insignificant (p-value>0.05). Another study found that Variant homozygous TT and heterozygous CT genotypes were significantly increased in diabetic groups than controls (p = 0.003) [2].

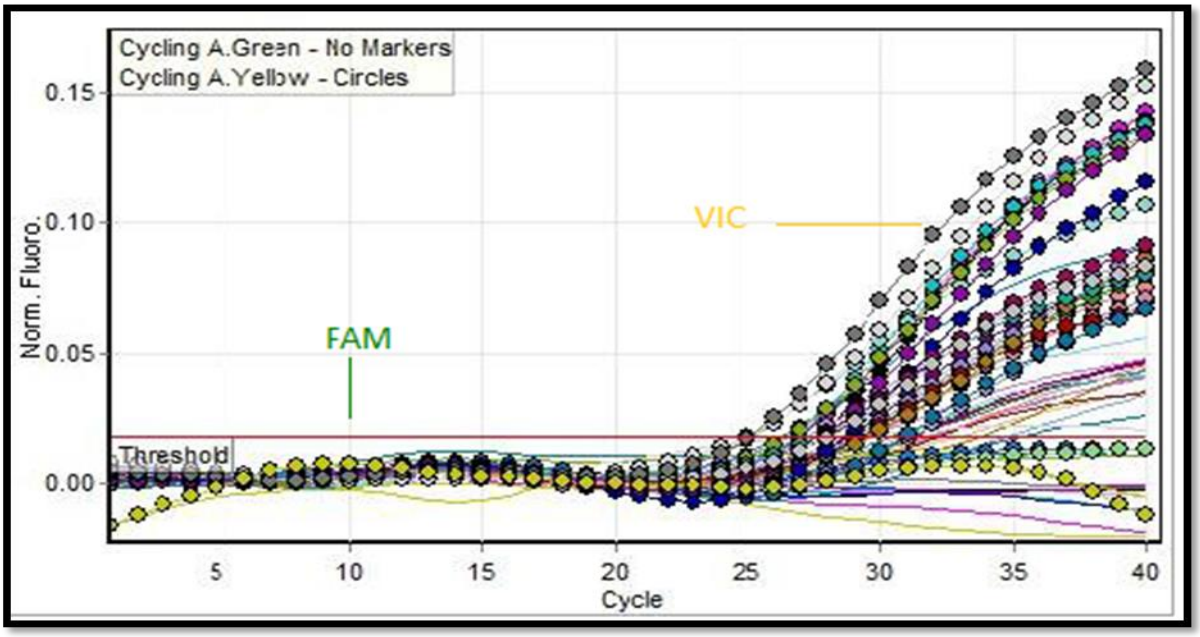

Figure 1. Wild (CC) and Mutant (TT) characters of TCF7L2 gene rs7903147 polymorphism in all samples.

CONCLUSION

The T allele of rs7903146 polymorphism of TCF7L2 confers susceptibility to the development of T2DM in the Iraqi population.

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