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Association of *Tcf7l2* Gene Expression and Polymorphisms with Type 2 Diabetes Mellitus Incidence in A Sample of Iraqi Population

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Abstract

The current study investigates the association of the gene expression and Polymorphism of the *TCF7L2* gene variations (rs78025551 and rs7903146) with increased incidence of type 2 diabetes mellitus (T2DM) in Iraqi patients. The study included 200 participants; 100 were T2DM patients, and 100 were healthy controls. Sex, age (divided into three groups), BMI, fasting blood glucose (FBG), and HbA1c % were investigated. Results indicated that there were substantial variations between patients and controls ($p < 0.01$) regarding age, age group, BMI, FBG, and HbA1c %, while no significant differences at the sex level. The first part of the research revealed the determination of *TCF7L2* gene expression. The median of gene expression (ΔCt) of the *TCF7L2* gene in patients' was (9.36), compared to the control (10.14); statistically, there were no significant differences ($p = 0.2$) between the studied groups. Moreover, the median of fold change in gene expression ($2^{-\Delta\Delta Ct}$) revealed up-regulation at (1.54). In the second part, two SNPs with polymorphic frequencies (rs78025551, [C/G] and rs7903146 [C/G/T]) were assigned in the DNA sequence of the PCR-amplified region (943 bp). It was found that highly statistically significant differences between T2DM patients in all genotypes and alleles of rs78025551 and rs7903146 in comparison with controls. The data pertaining to rs78025551 revealed notable genotype frequencies; there was a significant difference between genotypes GG in T2DM patients in comparison with control groups ($p < 0.049$). T2DM patients had a 4% GG genotype and an odd ratio of 8.60 (1.18-62.67). T2DM can manifest when people possess a homozygous mutant GG. The SNP's G allele was found in (24%) of T2DM patients and (17%) of healthy controls with a p-value of 0.01 (OR = 1.35, 95%CI = (0.95- 2.49)) suggesting a potential association with T2DM. This study identified an additional single nucleotide polymorphism (SNP) that exhibited association with T2DM. The study revealed a notable correlation between the rs7903146 single nucleotide polymorphism (SNP) and the occurrence of T2DM disease in individuals possessing heterozygote (CT) genotypes. The rs7903146 genotype frequency in the T2DM and control groups was 60% and 45%, respectively. The genotype of rs7903146, specifically the heterozygous CT genotype, exhibited a significant association with T2DM. The odds ratio (OR) for the CT genotype was 2.63. The SNP's T allele was detected in (48%) of T2DM patients and (32.5%) of healthy controls, with a *p-value* of 0.006.

Keywords: Polymorphism, rs7903146, rs78025551, *TCF7L2* , Type 2 Diabetes Mellitus

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مع حدوث داء السكر النوع الثاني في عينة من *TCF7L2* علاقة التعبير الجيني والتغاير الوراثي لجين المجتمع العراقي

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الخلاصة

تم اجراء هذه الدراسة للتعرف على التعبير الجيني وكذلك تعدد أشكال النوكليوتيد المفرد (rs78025551 SNPs و rs7903146) لموروث *TCF7L2*. وحدوث وتطور مرض السكري من النوع الثاني (T2DM) لدى المرضى العراقيين. تضمنت الدراسة 200 مشارك، 100من مرضى السكري النوع الثاني (T2DM)، و 100 من الاصحاء. أضافة الى ذلك تم فحص علاقة الجنس، والعمر (الذي قسم إلى ثلاث فئات عمرية)، ومؤشر كتلة الجسم (BMI)، وتحليل سكر الدم الصائم (FBG)، و تحليل السكر التراكمي (HbA1c٪). وأظهرت النتائج وجود فروق ذات دلالة احصائية في مرضى السكري النوع الثاني (T2DM) مقارنة بمجموعة السيطرة ($p < 0.01$) فيما يتعلق بالعمر والفئات العمرية ، ومؤشر كتلة الجسم (BMI)، وتحليل سكر الدم الصائم (FBG)، و تحليل السكر التراكمي (HbA1c٪)، من ناحية اخرى عدم وجود فروق ذات دلالة احصائية على مستوى الجنس. كان متوسط التعبير الجيني ΔCt للموروث *TCF7L2* في عينات الدم للمرضى (9.36) مقارنة بمتوسط ΔCt من المجموعة الضابطة (10.14). من الناحية الإحصائية، لم تكن هناك فروقات معنوية ($P = 0.2$) بين المجموعات المدروسة. علاوة على ذلك، كشف متوسط التغيير في التعبير الجيني المضاعف ($2^{-\Delta\Delta Ct}$) للموروث *TCF7L2* تنظيماً اعلى عند مستوى (1,54). في التنميط الجيني تم إجراء تسلسل الحمض النووي منقوص الاوكسجين لقطعة بلغ حجمها 943 زوج قاعدي ، تم العثور على اثنان من تعدد الأشكال النوكليوتيد المفرد (rs78025551, [C/G] and rs7903146 [C/G/T])، وأظهرت النتائج فروقات ذات دلالة إحصائية عالية في جميع الأنماط الجينية وترددات الأليل بين مرضى السكري النوع الثاني T2DM)) مقارنة مع عناصر التحكم. حيث اظهرت النتائج النمط الوراثي *TCF7L2* / rs78025551 أن هناك فرقًا كبيرًا بين الأنماط الجينية GG (متماثل الزيجة) في مرضى السكري النوع الثاني مقارنة بالمجموعات الضابطة (1.18−) =OR = 8.60, 95%CI (62.67), وكان أليل G له ارتباط كبير مع المرضى بقيمة ($P < 0.01$, $p \leq 0.049$). كشفت الدراسة عن علاقة ملحوظة بين تعدد أشكال النوكليوتيد المفردة (rs7903146 SNP) وحدوث مرض السكري النوع الثاني لدى الأفراد الذين يمتلكون الأنماط الجينية غير المتجانسة (CT). حيث كانت نسبة الاحتمالات (O.R) للنمط الجيني (CT)) مختلف الزيجة) (OR = 2.63)، وكان أليل T له ارتباط كبير مع الأفراد الذين تم تشخيص إصابتهم بمرض السكري النوع الثاني ، بقيمة ($P < 0.001$). مما يشير إلى ارتباط وثيق مع مرض السكري النوع الثاني.

1. Introduction

Diabetes mellitus (DM) is a multifactorial metabolic illness marked by chronic hyperglycemia and abnormalities in the metabolism of carbohydrates, proteins and fats due to deficiencies in insulin action, secretion, or both. DM can cause long-term damage as well as organ failure and malfunction. DM can cause thirst, polyuria, blurred eyesight, and weight loss, among other symptoms. In its most extreme forms, non-ketotic hyperosmolar states, or ketoacidosis, can occur and cause stupor, coma, and, in the absence of effective treatment, death [1]. DM is a chronic multifactorial metabolic disease with hyperglycemia resulting from insufficient secretion of insulin and/or resistance to insulin peripheral actions or both [2]. There are three main types of diabetes: type 1, type 2, and gestational diabetes. Type 1 diabetes (T1D) impacts individuals of all ages, both adults and children, which is characterized by the pancreas' inability to produce insulin as a result of either the death of pancreatic beta cells or their inactivity. Individuals affected rely on daily insulin shots to

sustain normal blood glucose levels. The aetiology of T1D remains incompletely elucidated, yet, researchers argue that both genetic and environmental factors contribute to its development [3].

Gestational diabetes is a form of diabetes that is initially identified during pregnancy. Gestational diabetes is diagnosed in approximately eight out of every 100 expectant women in the United States. Insulin function may be impaired by weight gain and hormonal fluctuations during pregnancy, leading to elevated blood sugar levels. Women who have experienced gestational diabetes have a 40-60% likelihood of developing type 2 diabetes within the next 5 to 10 years, despite the fact that this form of diabetes typically resolves after pregnancy [4].

Diabetes type 2, also known as non-insulin dependent diabetes mellitus (T2DM or NIDDM), is the predominant form of diabetes, often manifesting in adulthood. Due to rising obesity rates and sedentary lifestyles, adolescents and young adults are increasingly being diagnosed with diabetes type 2 or its precursor. In DM type 2, adipose, muscular, and hepatic cells have inadequate responsiveness to insulin. Insulin resistance is the term used to describe this phenomenon. Consequently, glucose is unable to penetrate these cells for energy storage, resulting in its accumulation in the bloodstream. Insulin resistance is a progressive condition that evolves gradually over time [5].

Type 2 diabetes mellitus (T2DM) constitutes a critical public health issue, with incidence escalating markedly worldwide. T2DM is a general term for diseases that are associated with pancreatic β-cell dysfunction, causing rising insulin secretion to offset insulin resistance in the peripheral tissues [6]. There are genes that considerably raise the incidence of T2DM, according to research conducted by the Genome Wide Association Studies (GWAS) [7]. For a more comprehensive understanding of the pathogenesis of the disease. A genetic approach will assist in highlighting the underlying causes of T2DM and may provide new insights for diagnostic treatment and prevention. Mutations in genes that encode proteins involved in insulin regulation and glucose homeostasis are prime candidates for T2DM. An effective method for detecting such problems is establishing a notable correlation between DM and a functional variation in a candidate gene. This is often accomplished by comparing a random sample of unrelated T2DM patients with a corresponding control group. This method may reveal a polymorphic allele with elevated frequency in the patient group, and such a notable connection might indicate a locus associated with illness susceptibility [8].

One of the most reproducible risk genes for diabetes identified across different ethnic populations is transcription factor 7 Like 2 (*TCF7L2*). T2DM has identified several SNPs in genes predisposing to type 2 diabetes. These genetic variants were repeated in several studies on different populations, confirming numerous associations [9]. The *TCF7L2* gene is a transcription factor that affects the expression of several genes; it thus performs various functions within the cell. *TCF7L2* gene is found on chromosome 10 q 25. 2–q25. 3 and consists of 19 exons [10, 11]. Being a member of the TCF family, the *TCF7L2* gene possesses a bipartite transcription factor to get involved in the regulation of various biological pathways, including the wingless-type MMTV integration site family (Wnt signaling pathway) [12]. The expression of multiple transcription factors is directly regulated by *TCF7L2* [13]. *TCF7L2* gene expression is increased in adipose tissue, and it is associated with lipid metabolism and glucose levels. Variations in *TCF7L2* expression may reduce the production of GLP 1, affecting insulin secretion and playing a role in Type 2 diabetes [14]. The incidence of SNPs in this gene is primarily implicated in the predisposition to T2DM and gestational diabetes mellitus, as well as other diseases [15, 16]. The effect of *TCF7L2* on

glucose metabolism also occurs in other organs like the stomach, brain, liver, and structural muscles [17]. The current investigation aims to illustrate the gene expression and genetic polymorphism of the *TCF7L2* gene variations (rs78025551 and rs7903146) with increased incidence of T2DM in Iraqi patients.

2. Methods

2.1. Study group

A case-control study encompassed 100 patients and 100 controls. The one hundred T2DM patients were selected from people attending the National Centre for Diabetes, Mustansiriyah University, Baghdad, Iraq, based on Ethical approval. The investigation was conducted in accordance with the ethical guidelines outlined in the Declaration of Helsinki. Prior to sample collection, both verbal and analytical consent from the patient were obtained. On May 29, 2024, a local ethics committee reviewed and approved the study protocol, consent form, and subject information (registration number CSEC/0524/0043). Both groups were divided into 50 males and 50 females. The patients included in this study met the diagnostic criteria for T2DM as outlined by the American Diabetes Association (ADA). The ADA standard was used to categorize T2DM: hemoglobin A1c (HbA1c) levels above 6.5% are indicative of diabetes, whereas levels ranging from 5.7% to 6.4% indicate pre-diabetes. Test results below 5.6% are considered normal. Fasting blood glucose (FBG) levels of 120 mg/dl (7.0 mmol/l) or higher means that the patient has diabetes mellitus [18]. In addition, sex, age, and body mass index (BMI) were also enrolled in the study. The study excluded a sample under 18 years old and patients with insulin-dependent diabetes mellitus. The controls were selected among subjects who were healthy in terms of non–diabetic and had no other endocrine disorders or chronic diseases. Also, they had no history of alcohol drinking.

2.2. Blood Collection

Five ml of blood samples were collected from each subject and directly divided into three parts: Part one: 2.5 ml put in a plain tube for serum collection and biomarker determination. Part two: (0.5 ml) put in the TRIzol TM Reagent tube for RNA extraction for gene expression analysis by reverse transcription quantitative polymerase chain reaction (RT-qPCR) analysis. Part three: (2 ml) put in the EDTA tube for DNA extraction and detection of SNPs by sequencing.

2.3. HbA1c and FBG measurement

AFIAS-1 immunoassay analyzer (Boditech, Korea) used in the measurement Of HbA1c and FBG Glycated hemoglobin (HbA1c%) was calculated using the Boditech kit, Korea, and the fasting blood glucose (FBG) test using the Biosystem kit, Spain [19].

2.4. TCF7L2 Gene Expression

In this study, RT-qPCR method was used to evaluate the expression of the *TCF7L2* gene after extracting total RNA using TRIzol^{TM} Reagent (Thermo Scientific, USA), following the manufacturer's instructions. A Quantum Fluorometer (Promega, USA) was used to detect the concentration of extracted RNA and the quality of samples for downstream applications. The separated RNA was reversely transcribed into complementary DNA (cDNA) using the GoTaq® one-step RT-qPCR System kit (Promega, USA). The Premier 3 software was utilized to design RT-qPCR primers, the annealing temperature and primer lengths displayed in Table (1), *β-Globin* was utilized as a housekeeping gene.

Table 1: Primers used for relative quantification of *TCF7L2* and *β-Globin* gene expression by RT-PCR.

Primer Name	5`-Sequence-3`	Annealing Temperature (ºC)
β-Globin-F	ACACAACTGTGTTCACTAGC	65
β-Globin-R	CAACTTCATCCACGTTCACC	
TCF7L2 -F	GGGTTGGAGGTTGGACAAATA	60
TCF7L2 -R	CAGATGTGAGAAGAGTGTCTGG	

The reaction mixture was modified to a total volume of 10 µl, following the manufacturer's recommendation. It consisted of 5 µl qPCR Master Mix (1X), 0.25 µl RT Mix, 0.25 µl $Mgcl_2$ for 1-Step RT-qPCR (1X), 0.5 µl of each primer (10 µM), 2.5 µl nuclease-free water, and 1 µl RNA (1–640 ng/µl). The mix was transferred to an RT-qPCR machine (BioMolecular System, Australia) that was programmed with the following optimal cycles: The cDNA synthesis was performed for 15 minutes at 37°C (one cycle), followed by an initial denaturation step for 5 minutes at 95°C (one cycle), followed by 40 cycles of denaturation at 95°C for 20 seconds, annealing at 60-65°C for 20 seconds, and extension at 72°C for 20 seconds. Finally, a melt curve was performed at 65-90 °C for one cycle. The ΔΔCt method was used to normalize the expression data for *TCF7L2* (the target gene) versus *β-Globin* (the housekeeping gene), and the results were presented as folding changes in gene expression [20, 21]. The CT value difference (ΔCt) between the target gene and housekeeping genes was calculated for each sample, and the fold-change in gene expression was estimated using the following formula:
Fold change = $2^{-\Delta\Delta Ct}$ [22].

2.5. DNA extraction and SNP genotyping

Genomic DNA was extracted from peripheral blood samples of both patients and controls using the manufacturer's technique (Relia PrepTM Blood gDNA" Miniprep System, Promega). The purity and concentration of the isolated DNA were assessed using a quantum fluorometer. In order to amplify the DNA segment, primers were designed in this study for the genotyping of *TCF7L2* gene SNPs (rs78025551 and rs7903146). Utilizing Geneious Prime software, Macrogene Company (Korea) produced the primers in a lyophilized form for the genes used in the investigation, as stated in Table 2.

Table 2: The Sequence of primers designed in this study.

Primer Name	Sequence 5`-3`	Annealing Temp. (ºC)
TCF7L2 -F	TGTAAAACGACGGCCAGTAGAGAGTGAAGGGAGATGAG	60
TCF7L2 -R	CAGGAAACAGCTATGACCCATCCACAAGCTAACACTAA	

2.6. Polymerase chain reaction

The PCR reaction was performed in a final volume of 25 µl, which composed of 12.5 µl GoTaq green Master mix (2X), 7.5 µl nuclease-free distilled water, 1 µl of each primer Forward and Reverse (10 µM), and 3 µl DNA sample (20-29 ng). After PCR amplification, agarose gel electrophoresis was adopted to confirm the presence of amplification. The PCR reaction was utterly dependent on the extracted DNA criteria. The reaction was carried out under ideal PCR conditions, with an initial denaturation at 95 °C for 5 minutes in one cycle, followed by 30 cycles of denaturation, annealing, and extension. Each denaturation cycle lasted 30 seconds at 95 °C, annealing cycles lasted 30 seconds at 60 °C. and the extension

cycles lasted 30 seconds at 72 °C. The ultimate extension procedure was accomplished with a single cycle at 72 °C for 7 minutes.

2.7. PCR products sequencing

PCR genotyping results were validated by sequencing randomly selected samples. The amplified *TCF7L2* locus was sequenced by Sanger's ABI3730XL di-deoxy chain terminating method using a sequencing kit (BigDye Terminator v3.1, Applied Biosystems, Foster City, CA, USA). Sequences were analyzed to determine the genotype using sequencing Geneious software version 5.2 (Applied Biosystems, Foster City, CA, USA). *TCF7L2* SNPs DNA sequences are available in the National Center for Biotechnology Information (NCBI).

3. Statistical Analysis

Data analysis was conducted using the available statistical package SPSS-26 and Win Pepi software. The data were presented using frequency, percentage, median (minimum-maximum values), mean, and standard deviation. For qualitative data folding of gene expression analyzed using Shapiro-Wilk and Kolmogorov-Smirnov tests). The levels have been given as (median and range). For quantitative data (sex, age, BMI, FBG, and Hba1c), analyzed and had been given a standard deviation and mean for a level as the data. Significant $P\ value \geq 0.05$ was used to assess deviations from the Hardy-Weinberg equilibrium for all genetic variations. WinPepi software estimated the odds ratio and 95% confidence interval for the genotypes and allele frequencies.

4. Results and Discussion

4.1. Demographic characteristics of T2DM

The results revealed that the means of sex, age, BMI, FBG, and HbA1c % levels were 50 male and 50 female, 56.3±7.9 years, 31.4±5.4 kg/m2, 199.78±84.76 mg/dl and 9.08±1.72 % for the diabetic patients, respectively, In contrast 50 male and 50 female, 45.0±12.6 years, 28.5±4.1 kg/m2, 100.04±16.67 mg/dl and 5.41±0.24 % for the controls, respectively. as shown in Table (3).

Table 3: Demographic characteristic of type 2 diabetes mellitus patients and control.

Characteristic		Group		*p-value*
		Controls N=100	Patients N = 100	
Sex	male	50	50	N. S
	female	50	50	N. S
Age Group	<50 years	62 (62.0)	16 (16.0)	0.01*
	50-60 years	25 (25.0)	54 (54.0)	
	>60 years	13 (13.0)	30 (30.0)	
	mean± S.D.	45.0±12.6	56.3±7.9	
BMI (kg/m²)	mean± S.D.	28.5±4.1	31.4±5.4	<0.001***
FBG (mg/dl) N.R. (80-120 mg/dl)	mean± S.D.	100.04 ±16.67	199.78 ±84.76	<0.001***
HbA1C (%) N.R. (<5.7%)	mean± S.D.	5.41±0.24	9.08±1.72	<0.001***

N.S= non-significant, N.R. = Normal rang , Percentage , S.D. = Standard Deviation, *p-value*= Probability value, *= Significant (<0.01), ***= Highly significant(<0.001).

The subjects were divided into three groups according to age: (<50) years, (50-60) years and (>60) years. In these age categories, the prevalence of cases was 16 (16%), 54 (54%) and 30 (30%) respectively. In contrast, According to Table 3, there were 62 (62%), 25 (25%) and 13 (13%) control participants for the same age groups, respectively.

The results shown in Table 3 indicated that there were substantial variations between patients and controls ($p < 0.01$) regarding age, age group, BMI, FBG, and HbA1c , while no significant differences were seen with sex between studied groups.

The association of age and body mass index (BMI) with the risk of T2DM has been examined in several studies. Ohno *et al*. found that the risk of developing T2DM increased steeply after BMI exceeded approximately 20-21 kg/m2 [23]. Jung *et al.* analyzed data from the Korean National Health Insurance Service-Health Screening Cohort and found that being obese before the age of 50 increased the risk of developing T2DM in the future [24]. However, it is important to note that the association between BMI and T2DM may vary across racial/ethnic groups. This finding is supported by meta-analyses and population-based studies demonstrating a strong correlation between BMI and the prevalence of DM [25, 26].

Strings *et al.* found that the association between BMI and T2DM was weaker among Black individuals, indicating that BMI may not be a reliable predictor of T2DM in this population [27]. Overall, maintaining a healthy BMI and avoiding obesity may help reduce the risk of developing T2DM, especially at a younger age. Younger age at diagnosis of T2DM is associated with a higher risk of both Alzheimer's disease (AD) and vascular dementia (VD) [28].

Screening criteria for T2DM may need to be revised to include younger and leaner adults, as a non-negligible proportion of new T2DM patients are younger than 35 years and have a normal body mass index (BMI) [29]. The association between obesity and T2DM is reduced with aging, indicating that older people may gain fewer potential benefits from weight loss interventions [30]. Age is a significant factor in the incidence of DM, while sex does not show a significant relationship [31]. In conclusion, a highly significant difference in BMI indicates an association between elevated BMI and diabetes prevalence.

FBG and HbA1c are both important markers for assessing glycemic control in T2DM. Several studies have investigated the relationship between FBG and HbA1c.

Hassan *et al.* showed that the levels of FBG and HbA1c increased in T2DM patients than in healthy controls with significant differences ($p<0.05$) [32]. Glycemic control, as measured by HbA1c levels, is important in reducing the probability of harmful cardiovascular events in individuals diagnosed with T2DM and coronary heart disease (CHD) [33].

HbA1c is a key marker used to manage blood glucose levels in individuals diagnosed with T2DM [34, 35]. It is a form of hemoglobin that represents the blood glucose level over some time [36]. Al-Attaby and Al-Lami observed elevated FBG and HbA1c levels in T2DM patients than controls [37]. The findings of the current study align closely with numerous previous studies investigating the relationship between FBG and hemoglobin A1C (HbA1C) levels in patients with DM.

4.2. Gene expression analysis of TCF7L2

After the RNA extraction, cDNA was synthesized using reverse transcription. The annealing temperature and melting temperature were set at (60 °C and 74.18 °C) respectively, for our target gene *TCF7L2*. The gene expression of *TCF7L2* was measured by RT-qPCR; the level of gene expression was adjusted to the level of a housekeeping gene and quantified using the folding ($2^{-\Delta\Delta Ct}$) and ΔCt values, as stated in Figure (1).

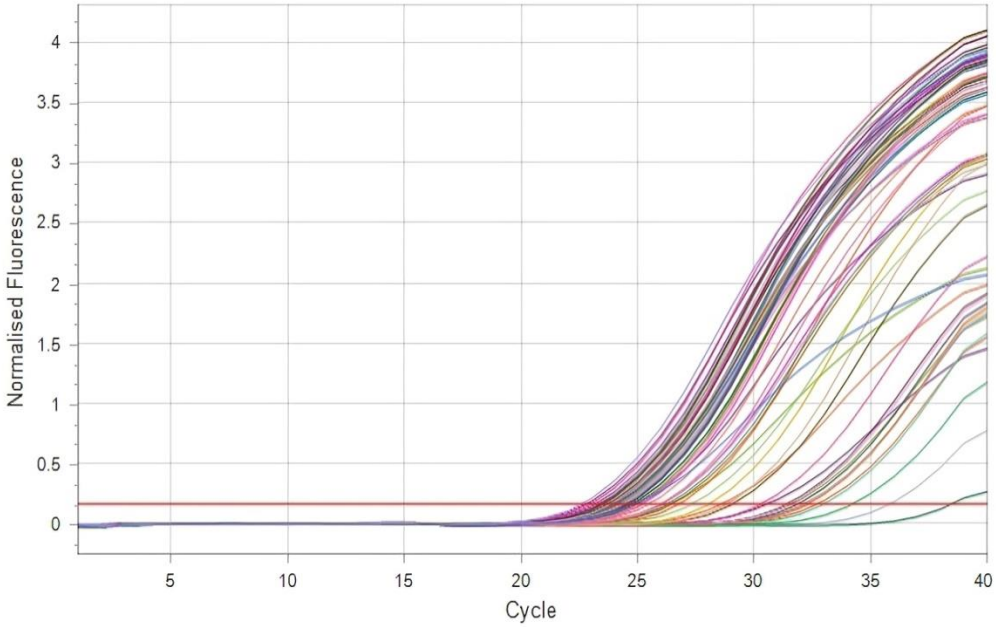

Figure 1: Amplification plots of *TCF7L2* gene by magnetic induction cycler (mic) RT-qPCR.

The median gene expression (ΔCt) of the *TCF7L2* gene in patients was (9.36), compared to the control (10.14); statistically, there were no significant differences ($p = 0.2$) in ΔCt for the *TCF7L2* gene between the two groups, as shown in Table (4). Moreover, the median of fold change in gene expression ($2^{-\Delta\Delta Ct}$) revealed up-regulation at (1.54).

Expression of the *TCF7L2* gene varies in individuals with T2DM; previous research has revealed that *TCF7L2* gene polymorphisms increase the risk of T2DM [38]. *TCF7L2* inhibits WNT/β-catenin signaling in subcutaneous abdominal adipose tissue and general metabolism [39]. Li *et al.* investigated the relationship between chromatin state and the genes involved in glucose metabolic processes [40]. *TCF7L2* gene expression is increased in adipose tissue, and it is associated with lipids metabolism and glucose levels. Variations in *TCF7L2* expression may reduce the production of GLP 1, affecting insulin secretion and playing a role in Type 2 diabetes [14]. In the Asian Indian population, the expression of the *TCF7L2* gene was significantly up-regulated in individuals with pre-diabetes and T2DM compared to those with normal control [41].

These studies have indicated that *TCF7L2,* with multipotent roles in the pathogenesis of T2DM and its gene expression, can be affected by its genetic and adipose tissue-related impacts [42].

Table 4: Expression folding ($2^{-\Delta\Delta Ct}$) of *TCF7L2* gene in type 2 diabetes mellitus patients and control.

Group	Controls (N=100) Median (min-max)	Patients (N=100) Median (min-max)	*p-value*
ΔCT	10.14(5.89-14.06)	9.36(5.45-17.96)	0.2 N. S.
$2^{-\Delta\Delta Ct}$	1.00(1.00-1.00)	1.54(0.00-23.32)	-

P-value= Probability value, Folding =$2^{-\Delta\Delta CT}$, N=Frequency, N. S.= Non-significant, min=Minimum, max= Maximum.

4.3. TCF7L2 gene SNPs

Two SNPs with polymorphic frequencies (rs78025551, [C/G] and rs7903146 [C/G/T]) were assigned in the DNA sequence of the PCR-amplified region (943 bp), as shown in Figure 2 and Table 5.

The sequencing result of rs78025551 SNP was observed in Figure 2-A, which had C/G genotypes. The study examined the alleles and genotype frequencies of the *TCF7L2* SNP (rs78025551) in Iraqi patients with T2DM and healthy controls. It found more heterozygotes than expected, indicating that the (rs78025551) SNP may be significant for T2DM development. In addition, the study discovered that T2DM patients had higher levels of heterozygote and homozygote mutant types (CG and GG), respectively, and lower levels of homozygote mutant type (GG). The results suggest a significant difference between genotypes GG in T2DM patients and control groups ($p \leq 0.049$), as mentioned in Table 5.
Four percent of T2DM patients had GG genotype and an odd ratio of 8.60 (1.18-62.67). Fisher's exact possibility assessment of a relationship's significance was chosen since it allows for possibility correction and is unaffected by small numbers (less than 5). The disease can manifest when people possess a homozygous mutant GG (odd ratio of GG was 8.60). The SNP G allele was found in (24%) of T2DM patients and (17%) of healthy controls; frequency differences between the two groups were statistically significant (OR = 1.35, 95%CI = (0.95-2.49), $p \leq 0.01$; Table 5). The (rs78025551) SNP distributions did not significantly deviate from HWE ($P = 0.346$) in patient groups, while it was in the control group (P=0.040). This may be due to the small sample size of cases under study, as shown in Table 5.
This study identified an additional single nucleotide polymorphism (SNP) that was observed in Figure 2-B, which exhibited an association with T2DM. The study revealed a notable correlation between the rs7903146 single nucleotide polymorphism (SNP) and the occurrence of T2DM disease in individuals possessing heterozygote (CT) genotypes. The genotype frequency in the T2DM and control groups was 60% and 45%, respectively. The genotype of rs7903146, specifically the heterozygous CT genotype, exhibited a significant association with T2DM. The odds ratio (OR) for the CT genotype was 2.63 (95% [CI] = 1.43 – 4.86, *p-value* = 0.003), as illustrated in Table 5.

Furthermore, it was observed that allele T had a substantial association with individuals diagnosed with T2DM, with a *p-value* of 0.006. The distribution of genotypes for the rs7903146 polymorphism in T2DM was shown to deviate from Hardy Weinberg equilibrium ($p = 0.043$), as shown in Table 5. The statistical significance of this link was evaluated using Fisher's Exact Probability.

To achieve equilibrium, five conditions must be met: a large population size, isolation from neighboring populations, absence of allele mutations (deletion or insertion), random mating, and the absence of natural selection. When the five conditions are not present, the process of evolution takes place, resulting in alterations in the allele frequencies within a population and the absence of Hardy-Weinberg equilibrium [43].

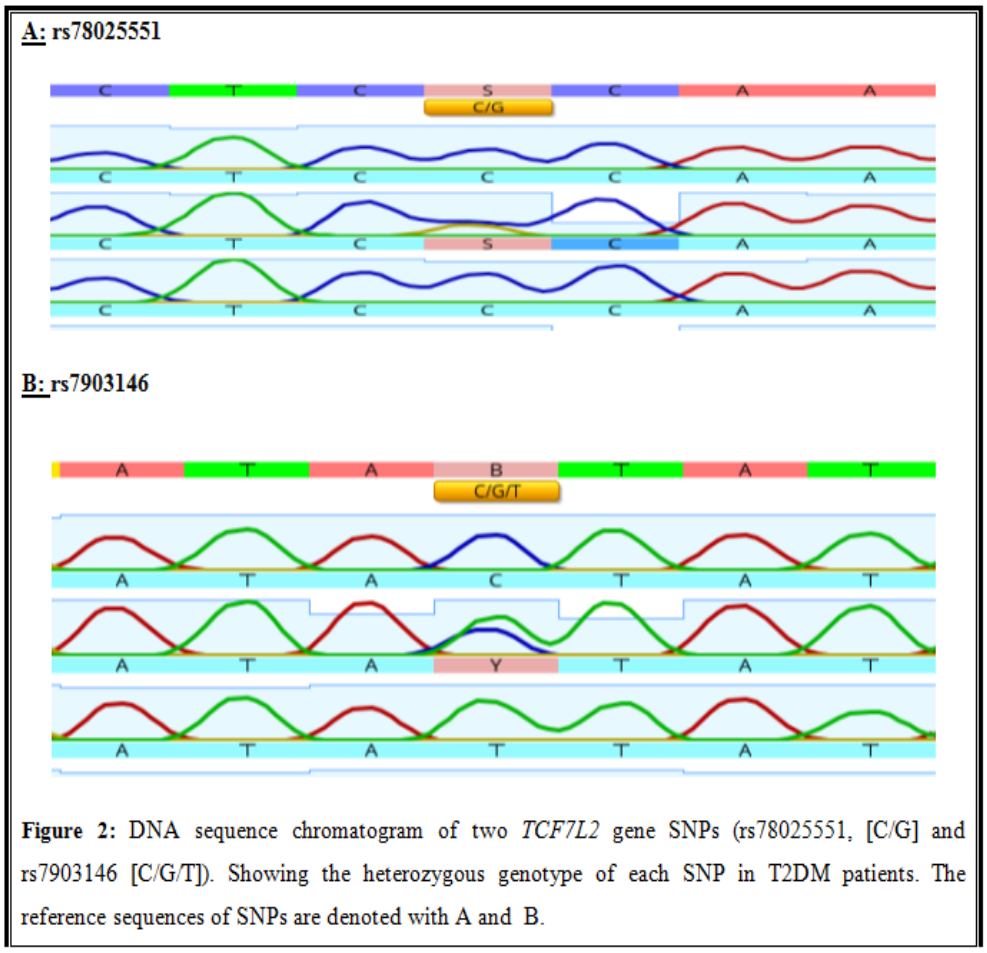

Figure 2: DNA sequence chromatogram of two *TCF7L2* gene SNPs (rs78025551, [C/G] and rs7903146 [C/G/T]). Showing the heterozygous genotype of each SNP in T2DM patients. The reference sequences of SNPs are denoted with A and B.

Table 5: Genotype and allele frequencies of SNPs of the gene *TCF7L2* and their HWE in patients and controls.

TCF7L2 gene SNPs Genotype and allele frequency	Patients N=100 (%)	Controls N=100(%)	*P*-value OR(95%CI)
rs78025551 genotype frequency			
CC	56(56%)	66(66%)	Reference
CG	40(40%)	34(34%)	0.303NS 1.39(0.75-2.58)
GG	4(4%)	0(0%)	0.049* 8.60(1.18 - 62.67)
HWE *P*–value	0.346NS	0.040*	-
rs78025551 allele frequency			
C	152(76%)	166(83%)	Reference
G	48(24%)	34(17%)	0.01* 1.35(0.95- 2.49)
rs7903146 genotype frequency			
CC	22(22%)	45(45%)	Reference
CT	60(60%)	45(45%)	0.003*** 2.63(1.43 - 4.86)
TT	18(18%)	10(10%)	-
HWE *P* -value	0.043*	0.797NS	-
rs7903146 allele frequency			
C	104(52%)	135(67.5%)	Reference
T	96(48%)	65(32.5%)	0.006*** 3.58(1.47 - 8.72)

Single nucleotide polymorphism (SNPs), confidence interval (CI), odds ratio (OR), Hardy-Weinberg equilibrium (HWE), Significant* ($P \leq 0.05$), Significant** ($P \leq 0.01$), Non-significant.

The current study demonstrated that *TCF7L2* rs7903146 (C/T) polymorphism was significantly linked with T2DM; however, no significant correlation was found for rs78025551 (C/G) polymorphism. This result agrees with Yazdi *et al.,* [44], which indicates, in addition to numerous studies, that polymorphisms are significantly associated with T2DM. The SNP rs7903146 of the *TCF7L2* gene is the most significant genetic marker linked to the risk of T2DM [45].

The *TCF7L2* gene encoding *TCF7L2* transcription factor has many functions based on the polymorphism and is, therefore, idealized as a pleiotropic gene. Carriers of the *TCF7L2* rs7903146 C/T variant show vulnerability to T2DM [46]. The C allele of the rs78025551 C/G polymorphism results in decreased susceptibility to gestational diabetes mellitus (GDM), while the T allele of rs7903146 increases the susceptibility to GDM in the Chinese Han population [47].

Thus, rs7903146 in the *TCF7L2* gene has been identified as a risk factor in T2DM. The T allele of rs7903146 is mostly associated with an increased risk of T2DM. However, the interaction between *TCF7L2* gene polymorphisms and susceptibility of T2DM might be different among various groups of populations. Collectively, the *TCF7L2* gene and the rs7903146 SNP are major genetic markers associated with T2DM in different populations [48-51]. The T2DM risk allele for rs7903146 lowers the adipose progenitors *TCF7L2* expression and increases adipose insulin sensitivity. As well as the modulation of pancreatic insulin secretion, genetic polymorphism of *TCF7L2* could produce the T2DM impact on the function of adipose progenitors [39].

T2DM patients have been found to have *TCF7L2* mutations, indicating that the gene may be the cause of this disease [52]. In addition, other single nucleotide polymorphisms, including *TCF7L2* rs7903146 and rs12255372 polymorphisms, have also been associated with T2DM in the Indian and Pakistani populations [53]. The results of these studies reveal that the *TCF7L2* genes seem to be involved in the T2DM pathogenesis and susceptibility of T2DM. Polymorphisms in *TCF7L2* have been investigated in subjects with T2DM. Various research works have established strong evidence for the role of *TCF7L2* gene polymorphism in susceptibility to T2DM [50, 54].

Mohib *et al.* found the most common variant in CC rs7903146 to be excessively high, present in the majority of the 38 sequences examined and in all of the samples from individuals without T2DM. Moreover, a tiny proportion of Bangladeshi T2DM patients found a less prevalent variant called CT, contradicting previous findings on the association between rs7903146 and T2DM [55].

Other investigations conducted in Iraq have demonstrated the association of *TCF7L2* gene polymorphisms with the risk of T2DM and its related complications. This study was conducted in the Kurdish population of Erbil province in Iraq, in which a positive association between *TCF7L2* rs7903146 SNP and T2DM risk was observed. This implies that the population is predisposed genetically to T2DM and that it is important to research what genes lead to the disorder [51].

In Al Najaf Governorate, the variant of *TCF7L2* gene rs12255372 SNP has been analyzed in Iraqi Arabic T2DM patients, and the possible contribution of this SNP in genetic susceptibility to T2DM of this population. This research helps to ascertain the genetic variation that contributes to the development of T2DM in Iraq, as well as the importance of personalized medicine and targeted intervention [56]. Maragheh *et al.* showed that the frequency of genotypes in healthy individuals was as follows: TT 33.7%, CT 49.5%, and CC

16.8%. In contrast, among diabetic patients were TT=43%, CT=43%, and CC=14%. T allele percentage in healthy and diabetic people was as much as 42.1% and 64.5%, respectively [57].

The frequency of the risk allele "T" was significantly higher in cases (30.7%) compared to controls (2.3%), and genotype "CT" was more commonly associated with diabetic cases than healthy controls with a $p<0.001$, revealing a strong association of *TCF7L2* gene polymorphism (rs7903146 [C/T]) with T2DM in the Chennai suburban populations [58].
In the Moroccan population, the *TCF7L2* gene (rs7903146 C/T polymorphisms) was shown to be significantly associated with an increased risk of type 2 diabetes. The genotype and allele distribution analysis revealed that the TT genotype was more prevalent in T2DM patients (24.0%) than in the healthy control group (5%) with a P-value < 0.0001. The T allele frequency was higher in diabetes patients (45.2%) than in healthy controls (34.5%), with a *P*-value = 0.005 [59].

The outcome of the study could potentially be influenced by the small sample size, expensive kits, and materials, given the absence of prior research conducted on this particular polymorphism within the Iraqi society.

CONCLUSION

According to the current study, *TCF7L2* gene is implicated in the Iraqi population in the development of T2DM, showing linkage with the development of T2DM as a result of the association of *TCF7L2* polymorphisms with T2DM risk. Genomic association studies in Iraq are crucial in establishing genetic markers for the adaptation of personalized medicine in the management of T2DM. The role of *TCF7L2* gene polymorphisms in T2DM in the Iraqi population is well-established. Further research is needed to elucidate the specific mechanisms underlying this association.

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