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TCF7L2 Gene Polymorphism rs290487 and Its Correlation with Insulin Resistance in Al-Najaf governorate

Dhurgham Abdul abbas Abed Al-Graiti

Dhurgham.algraiti@student.uokufa.edu.iq

Faculty of Pharmacy/University of Kufa Department of Clinical Laboratory Sciences

Haider F. S. Al-Zubaidy

Hayderf.salman@uokufa.edu.iq

Faculty of Pharmacy/University of Kufa Department of Clinical Laboratory Sciences

Abstract

Background:

T2DM is a worldwide health challenge manifested by insulin resistance (IR) and failure of β-cell function. Genetic variants, specially the rs290487 SNP in the *TCF7L2* gene, are highly associated to T2D occurrence in different populations. However, the occurrence and impact of this polymorphism on T2DM and linked metabolic problems in the Al-Najaf population remain non explored.

Objective:

The study goal is to explore the linking of the *TCF7L2* gene SNP rs290487 with T2D.

Materials and Methods:

A case-control study was done out on 200 participants (100 T2DM patients and 100 controls) aged 35–65 years. Samples of blood were collected and analyzed for phenotypic and genotypic analyses. Genotyping of the *TCF7L2* rs290487 SNP was carried out using PCR-RFLP, and biochemical parameters were analyzed. Statistical analysis was done using t-tests and ANOVA test.

SNP with T2DM in the Al-Najaf population and explore its influence on insulin resistance in T2DM patients.

Results:

This study focused to explore the linking between rs290487 SNP of the TCF7L2 gene and T2D in the AL-Najaf community. The study shows no significant alteration in age and body mass index (BMI) among the patient and control groups. However, fasting serum glucose (FSG) and insulin grade were highly increased in T2D patients (P<0.0001), lead to insulin resistance. HOMA-IR reading also view a significant elevation in patients (P=0.025). Genotyping analysis indicate a strong association between the T allele and T2DM, with TT genotype indicating the highest risk (OR=36.79, P<0.0001). The results underscore the importance of rs290487 in modulating T2DM risk. However, no significant association has been discovered between HOMA-IR and genotypes (P > 0.05).

Conclusion:

The TCF7L2 rs290487 TT genotype and T allele are linked to T2DM risk in Al-Najaf, highlighting the link between genetic predisposition and metabolic traits.

Introduction:

DM is a metabolic syndrome manifested by persistence elevation of serum glucose grade either fasting or postprandial [1]. This blood glucose elevation result from either failure of insulin secretion or from impaired its action or both [2]. Miscellaneous symptoms of diabetes mellitus include hungering, thirsting, frequent urination, frequent urinary tract infection, feeling of tiredness and the hardening of wounds healing. Also respected numbers of patient may not suffer from these symptoms and they are diagnosed accidently [3]. The global spread of DM and its associated problems make it a universal challenge. According to the IDF (international diabetes federation) 1 from each 11 adults suffering from diabetes and around 450millions patients in the world diagnosed with diabetes in 2015. The incremental increase in diabetes mellitus makes it epidemic and many factors complicating to make this increase including urbanization, increase age mean, economic growth, unhealthy food and lack of physical activity. Considerably 90% of cases of DM is diagnosed as type 2 diabetes mellitus [4]. Around 1 million subject suffering from T2DM in Iraq [5]. In Basrah south of Iraq a local study including 5400 volunteers aged from (19-94 years) find diabetes prevalence is 19.7% [6]. In chromosome 10q25.3 located the gene TCF7L2. It consist of 18 exon and located in different tissues [7]. TCF7L2 gene form a

transcription factors that play important roles in the Wnt signaling pathway, which affect directly in the insulin secretion & beta-cell function, also Wnt pathway affect on cell division, polarity and programed cell death during both embryonic development and adult cell balance. Wnt system dysfunction can lead to various human diseases [8]. According to the genome-wide association studies (GWAS) discovered many single nucleotide polymorphisms responsible for T2DM. These SNPs found to interfere with lipid metabolisms, insulin production, glucose metabolisms and insulin receptor connection [9]. Among many SNPs at TCF7L2 gene the rs290487 SNP shown strong association with T2DM in different cultures and ethnicities [10]. This shown impact of high risk of SNPs on T2DM will differ globally. Although the effect of the SNP rs290487 is approved on the presence of T2DM but its occurrence varies among the different population [11]. other intronic variant rs290487 is related with T2DM occurrence, with a slightly allele occurrence of about forty percent in the Chinese community [11]. While numerous researches have ensured the relation of rs290487 with T2DM [12, 13]. A few other researches have been found no relation with T2DM [14]. There are studies that found the rs290487 are associated with the elevation of occurrence insulin resistance [15], also other studies suggest that SNP rs290487 are associated with glucose metabolism un the liver [16]. The goal of this paper to proof the relation of TCF7L2 gene rs290487 SNPs & the risk of incidence of T2D in Al-Najaf community and to search the influence of TCF7L2 gene on insulin resistance in T2D patients.

Material and methods

Study design

This is a case-control study involving 200 participants, split into 2 groups: 100 patients with T2D and 100 healthy controls. The study will run from October 2023 to August 2024. Samples were gathered from the Al-Najaf Diabetic Center and analyzed in the laboratories of the faculty of Pharmacy at the Kufa university.

Sample collection

The patient group consists of 100 individuals diagnosed withT2DM, aged between 35 and 65. These participants were selected from the Diabetes Center in Al-Najaf Al-Ashraf, with diagnoses made by specialist physicians according to the inclusion criteria. The control group, also

comprising 100 individuals aged 35 to 65, was gathered from Al-Sader Medical City in Al-Najaf Al-Ashraf. The study excluded individuals with known diagnoses of type 1 diabetes mellitus, hypertension, heart disease, cardiovascular conditions, renal diseases, or any other significant health conditions.

After an overnight 5 mL of whole blood was gathered from each one via peripheral vein puncture. The sample was then divided into two parts. The first part, composed of 3 mL, was placed in a gel tube and leaved to coagulate at 25°C for 15 minutes. After centrifuging for ten min. at 2000 xg, the separated serum was moved to an Eppendorf tube & put in a deep freezer at -20°C for future phenotyping analysis. The remaining 2 mL was used for genotyping analysis, moved to an EDTA tube, and also packed in a deep freezer.

Genotyping measurement

DNA was separated from the sample utilizing a DNA purification kit (Addbio). The TCF7L2 gene and the single nucleotide polymorphism (SNP) rs290487 were examined utilizing polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) as detailed in Table 1. The analysis was then performed using agarose gel electrophoresis in conjunction with the PCR products. Specific primers and master mixes were used to amplify each SNP. Following amplification, PCR products were digested with specific restriction enzymes (BstU1) sourced from the Fermantas kit, and the resulting parts were isolated on a 2% agarose gel as described in Table 2 [17].

Table 1: The sequence of primers used for TCF7L2 gene amplification SNP (rs290487).

SNP	Primer
rs290487	F: 5′-AGG AGG CTG CCA TAT TTG TTT ACT T-3′ R: 5′-ACA CCT TTC TCA TTT TCA ATT TCG C-3′

Table 2: Program of PCR thermocycling conditions for TCF7L2 gene amplification

Phase	Tm (°C)	Time	Cycles
Initial denaturation	95°C	10 min	1X
Denaturation	95°C	30 sec.	35X
Annealing	55°C	30 sec.	
Extension	72°C	30 sec.	
Final extension	72°C	10 min	1X

The Ethical Committee Approval

The approval done from Ethical Committee (Scientific Committee for Research Ethics in Najaf Health Directorate) for the protocol of study done by document number 19187 at date 29/5/2024.

Result

The analysis of age values and body mass index values between the patient group and the control group indicated no significant differences, with P-values recorded at 0.56 and 0.26, respectively. Additional parameters were observed to differ in T2DM patients in contrast to healthy individuals. The patient group exhibited markedly significant increases ($p<0.0001$) in the levels of fasting blood and insulin when compared to the control group. In T2DM patients, the HOMA-IR were found to be significantly higher, with P-values of 0.025 compared to the control group. The analysis of normally distributed data, including age and BMI was conducted using the unpaired t-test, with results presented as mean ± SD. In contrast, non-normally distributed data, such as FSG, insulin, and HOMA-IR, were evaluated using the Mann-Whitney test, with findings reported as median (Q1, Q3). The biochemical parameters are presented in table 3.

Table 3: Values of Anthropometric and Biochemical Factors in T2DM and Control Groups.

Parameters	Mean± SD		P Value
	T2DM group (N=100)	Control group (N=100)	
Gender (M\F)	(69\60)	(53\45)	
Age (years)	48.81 ± 8.16	49.5 ± 9.17	0.56
BMI (kg/m2)	27.48 ± 3.53	26.92 ± 2.96	0.26
FSG (mg/dl)	183.87 ± 35.91	90.73 ± 10.77	<0.0001**
Insulin	6.73 ± 1.74	5.45 ± 0.97	<0.0001**
HOMA-IR	2.93 ± 0.76	2.68 ± 0.64	0.025*

Note: * = mean that a significant result.

Note: ** = mean that a highly significant result.

The PCR yielding were digested using the restriction enzyme BstU1. The wild-type genotype (CC) is characterized by the presence of two bands at 25 bp and 128 bp. The presence of three bands (153 bp, 128 bp, and 25 bp) confirms the heterozygous genotype (CT). Lastly, a single band at 153 bp indicates the homozygous mutant genotype (TT). The DNA dye used was RedSafe from Intro, Korea, and the gel concentration was 2%. The electrophoresis was done at 75 V for thirty minutes. The DNA ladder used is listed in Table 4 and shown in Figures 1 and 2

Table 4: Digestion product result for SNP rs290487 C/T of polymorphism in TCF7L2 gene.

Genotypes		No of band	Size of the band (bp)
Wild pattern	CC	1	153
Heterozygous pattern	CT	3	153, 128 and 25 bp
Homozygous pattern	TT	2	128 and 25 bp

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Figure 1: DNA checking step

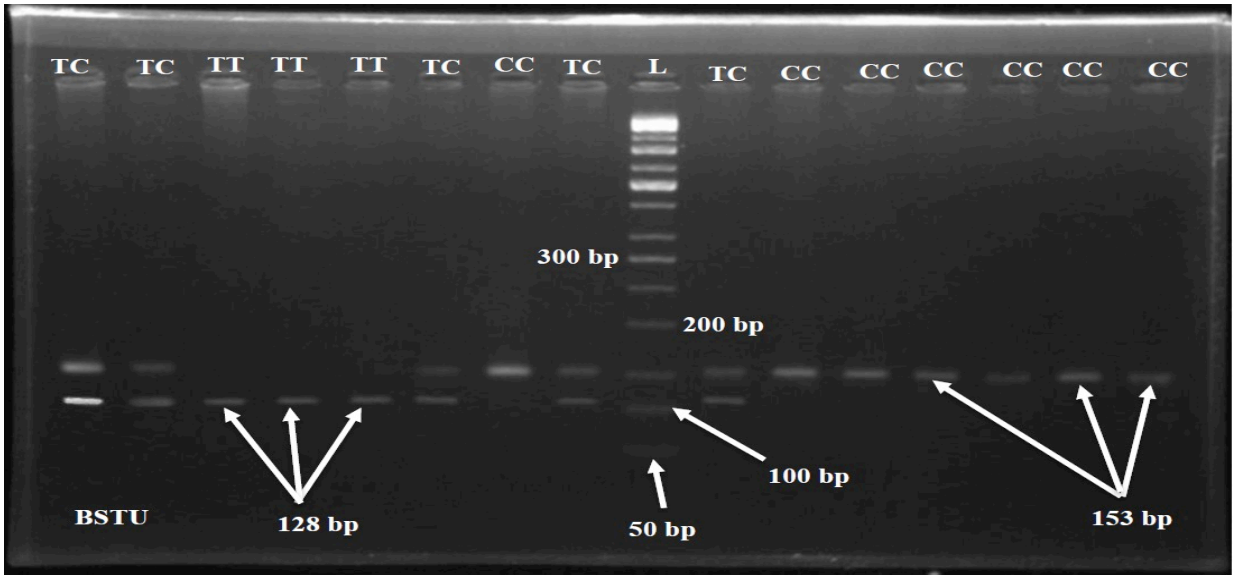

Figure 2 shows the RFLP product of the SNP rs290487 C/T of the TCF7L2 gene in the control group after digestion with the BstU1 enzyme. The product was then electrophoresed on 2% agarose for 45 minutes at 75 V and immediately visualized under UV light. M: DNA ladder, with one band measuring 153 bp for the wild type (WT) genotype (CC), and three bands measuring 153, 128, and 25 bp for the heterogenotype (CT) confirmed. Lastly, the gel expressing two bands of 128 and 25 bp for the mutant genotype (TT) was detected

Table 5 presents the results of SNP (rs290487 C/T) for T2DM and control individuals across several inheritance models. The codominant model indicated that patients with heterozygous genotypes (CT) exhibited a substantial increase (OR = 0.38, 95% CI = 0.29 – 0.51, P = 0.0001) compared to the control group. Patients with the homozygous genotype (TT) exhibited a substantial increase (OR=36.79, CI 95%= 5.23 – 285.65, P=0.0001) matched to the control group. The dominant model exhibited a significant increase in patients with (TT + CT) genotypes (OR=1.44, CI 95%=1.2 – 1.73, P=0.0001) relative to the control group. The recessive model demonstrated that patients with TT genotypes showed a significant increase (OR = 36.79 , 95% CI = 5.23 - 258.73, P = 0.0001) relative to the control group. The frequency of (T) in the sick group considerably increased (OR= 2.3, CI 95%= 1.83 – 2.9, P<0.0001) compared to the control group.

Table 5: Genotype and allele frequency results of SNP rs290487 C/T TCF7L2 gene in T2DM and control subjects.

	Patient group (N= 100)		**Control group (N= 100)**		**OR (CI %)** 153, 128, 25, bp	**P value**
	NO.	**%**	**NO.**	**%**		
Genotyping						
Codominant						
CC	15	26.79	41	73.21	Reference group	
CT	32	35.56	58	64.44	0.38 (0.29-0.51)	0.0001**
TT	53	98.15	1	1.85	36.79 (5.23-258.65)	0.0001**
Dominant						
CC	15	26.79	41	73.21	Reference group	
TT + CT	85	59.03	59	40.97	1.44 (1.2-1.73)	0.0001*
Recessive						
CC + CT	47	32.19	99	67.81	Reference group	
TT	53	98.15	1	1.85	53 (7.47-375.83)	0.0001**
Frequency						
C	62	30.69	140	69.31	Reference group	
T	138	69.7	60	30.3	2.3 (1.83-2.9)	< 0.0001
	200		200		400	

The ANOVA test was employed to analyse HOMA-IR values in connection to the genotypes of the SNP rs290487 C/T of the TCF7L2 gene. The SNP rs290487 in the TCF7L2 gene shows no significant association with the clinical parameters HOMA-IR (see Table 6).

Table 6: Results of phenotypic parameters of diabetic patients analyzed in relevance to the rs290487 C/T under the dominant model

Index BSTU	Genotype			P-value		
	CC	CT	TT	CCvsCT	CCvsTT	CTvsTT
Homa-IR	47.34 ± 10.4	48.69 ± 13.66	47.37 ± 11.3	0.78	0.99	0.71

Discusion:

Several recent studies have highlighted the link between TCF7L2 SNPs and T2DM in various ethnic groups. However, there is limited data on the linking of the rs290487 variant of TCF7L2 and T2D in the Iraqi population. The results from other cohorts have shown inconsistency. Therefore, we sought to investigate the TCF7L2 variations in relation to T2D and its metabolic traits in this specific population. We analyzed the allele and genotype frequencies of these polymorphisms in individuals having and have not T2D, examining their impact on anthropometric measurements and diabetes-related variables [18]. The anthropometric and biochemical features of individuals with type 2 diabetes and healthy controls were compared in this research. There were no notable changes seen in age and body mass index [19], However, T2D patients had noticeably higher fasting serum glucose (FSG) levels owing to insulin resistance and decreased insulin secretion caused by damaged pancreatic beta cells. These results are consistent with research that has linked beta-cell destruction to high blood sugar [20]. In the early stages of T2DM, insulin resistance and the compensatory overproduction of insulin by the pancreas result in significantly higher serum insulin levels. However, chronic insulin resistance eventually leads to hyperglycemia and pancreatic dysfunction. Patients with T2D showing greater HOMA-IR scores, indicating severe IR. This is a crucial predisposing factor for the resulting of T2D and its problems, such as nephropathy & cardiovascular disease. These findings

align with other studies that have associated hyperglycemia and HOMA-IR with an elevated risk of disease, underscoring the pivotal role of IR in the progression of T2DM [21, 22].

This study explores the SNP rs290487 C/T of the TCF7L2 gene in relation to T2D using a case-control approach. Previous research highlights this polymorphism as a significant genetic predisposing for T2D [7, 11]. Analysis viewed a strong relation between SNP rs290487 and T2DM in the Al Najaf population, linking this variant to disease occurrence. The study analyzed SNP rs290487 (C/T) genotypes in T2D patients and controls.

The **TCF7L2** gene is a well-established genetic factor linked to T2D. The SNP **rs290487** is associated with changes in gene activity that may influence insulin secretion and glucose metabolism. Table 5 likely displays the distribution of genotypes and alleles of **rs290487** in patients with T2DM (patient group) compared to healthy individuals (control group). The genotyping data presented in Table 5 offers a detailed comparison between the T2DM patient group and the healthy control group [11].

In the codominant model, the distribution of genotypes (CC, CT, and TT) shows notable differences between the two groups. The **CC genotype**, serving as the reference group, is significantly more common in the control group (73.21%) compared to the patient group (26.79%), suggesting that individuals with the CC genotype are less likely to develop T2DM. In contrast, the **TT genotype** is much more occurred in the patient group (98.15%) linked to the control group (1.85%), with an odds ratio (OR) of **36.79**, indicating a robust linking between the TT genotype and the occurrence of T2DM [18]. This demonstrates a very robust linking between the **TT genotype** and an elevated risk of T2DM. Although the **CT genotype** is less common in the patient group, it seems to offer a protective effect compared to the TT genotype, with an odds ratio (OR) of **0.38**. These data focusing on the potential role of **rs290487** in influencing T2DM risk by affecting genotype distribution [23, 24]. The dominant model, which combines the **TT** and **CT genotypes**, further strengthens the association between the **T allele** and T2DM. Individuals carrying at least one **T allele** (TT or CT) have significantly higher odds of being in the patient group, with an odds ratio (OR) of **1.44**. This finding highlights the cumulative effect of the T allele in increasing susceptibility to T2DM, even when present in a heterozygous state.

The **recessive model** provides an even clearer indication of the risk associated with the **TT genotype**. When compared to individuals with the **CC** or **CT genotypes**, those with the **TT genotype** have an OR of **53**, signifying an exceptionally high risk of developing T2DM. This suggests that homozygosity for the **T allele** is a main genetic elements in the appearance of T2DM [18].

The allele frequency data further supports these findings. The **T allele** is significantly more prevalent in the patient group (**69.7%**) compared to the control group (**30.3%**), with an odds ratio (OR) of **2.3**. This suggests that the **T allele** is strongly linked to the T2DM, while the **C allele** seems to play a protective role. The high statistical significance (**P < 0.0001**) across all models and comparisons emphasizes the strength and reliability of these results [25].

Biologically, the TCF7L2 gene is inserted in the controlling of insulin secretion & glucose metabolism, and SNP rs290487 may influence these processes. The increased frequency of the T allele and TT genotype in the patient group suggests that this variation may impair the normal function of TCF7L2, leading to a predisposition to T2DM. These findings have important implications for understanding the genetic basis of T2DM and for identifying individuals at risk [26].

The table 6 compares HOMA-IR across different genotypes (CC, CT, TT) of a specific SNP. The p-values assess the statistical significance of differences between the genotype groups for this index.

The analysis of the metabolic HOMA-IR reveals varying degrees of association with the genotypes CC, CT, and TT. For HOMA-IR, there is no important variation across the genotypes, as the p-values in whole comparisons are well above 0.05, suggesting that this SNP does not substantially influence insulin resistance [18].

Conclusion:

1-TCF7L2 rs290487 is highly linked with T2DM in the AL-Najaf community, with the TT genotype showing a significant link to elevation risk.

2-Insulin resistance and higher HOMA-IR scores were observed in T2DM patients, confirming their role in disease progression.

3-The T allele is more prevalent in T2DM patients, and the TT genotype shows an exceptionally high risk (OR = 36.79), while the CC genotype appears protective.

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