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The TCF7L2 rs7903146 Variant as a Predictor of Type 2 Diabetes Susceptibility in the Iraqi Middle Euphrates Region

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ABSTRACT

Background: *Type 2 Diabetes Mellitus (T2DM) is a complex metabolic disorder characterized by insulin resistance and pancreatic β-cell dysfunction. Certain populations have identified the TCF7L2 gene, particularly the rs7903146 single nucleotide polymorphism (SNP), as a major genetic risk factor. In this study, we aim to investigate the impact of the TCF7L2 rs7903146 polymorphism on T2DM in Middle Euphrates region of Iraq.* ***Methods****: A case-control study design was conducted with 300 total participants, 150 T2DM patients and 150 healthy controls. Genotypic data was collected using tetra-primer Amplification Refractory Mutation System-Polymerase Chain Reaction (ARMS-PCR) and biochemical parameters included fasting blood glucose, serum insulin levels, lipid profiles, and HbA1c. Statistical Analysis incorporated Hardy Weinberg Equilibrium, logistic regression, Bayesian statistics, and Bayesian analysis.* ***Results:*** *In our research, it seems that the rs7903146 SNP had no impact on the risk of developing T2DM in the participants. There was no significant difference in the T-Allele frequency of the polymorphism where case T2DM patients and controls T2DM (23 % vs. controls 20 %, p = 0.45). There were no significant differences on the studied diabetic and control groups regarding the allelic and genotypic distributions under all genetic models. Moreover, no significant difference in insulin resistance or lipid metabolic profiles was observed among the different genotypes of rs7903146, indicating that this SNP polymorphism may have limited impact on this local population.* ***Conclusion:*** *The information obtained about the middle Euphrates people suggests that rs7903146 polymorphism within TCF7L2 gene is not one of the main candidates factors for the* development of T2DM and its associated disorders in this population. The results emphasize the need to focus on specific features of the population of the patients being studied, including the genetic and environmental factors when investigate T2DM.

Keywords: TCF7L2, rs7903146, Type 2 Diabetes Mellitus, Genetic Association, Middle Euphrates, Metabolic Profile.

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INTRODUCTION

Type 2 Diabetes Mellitus (T2DM) is a long-lasting chronic condition characterized by increased blood sugar levels due to the body's resistance to insulin or insufficient insulin secretion (Fadheel et al., 2022). This is responsible for more than 90% of diabetes cases, and its prevalence has rapidly become a major global concern, with estimates predicting it will surpass 600 million cases in 2045, a steep increase from 425 million in 2017 (Forouhi et al., 2019). It leads to other conditions such as cardiovascular disease, nephropathy, neuropathy, retinopathy which increases overall morbidity and mortality (Rosengren et al., 2023).

In the case of T2DM, its causes are complex and include a mix of surrounding, behavioral, and hereditary elements (Algenabi et al., 2021). Of the various genetic factors, the

most consistently and strongly linked with T2DM across different populations is the TCF7L2 gene (Transcription Factor 7-Like 2), which is situated on chromosome 10q25.3 (Grant et al., 2006; Cauchi et al., 2007). TCF7L2 has critical impacts on the Wnt signaling pathway where processes like cellular proliferation, differentiation, and metabolic homeostasis are controlled. In pancreatic beta cells, TCF7L2 affects the mechanisms of insulin secretion and glucose sensing (Ip et al., 2012). Of the numerous single nucleotide polymorphisms (SNPs) discovered within this gene, rs7903146 (C>T), found within the third intron, is particularly noteworthy. The T allele of this SNP has been linked to a greater likelihood of developing Type 2 Diabetes Mellitus (T2DM) across multiple different ethnic groups (Lyssenko et al., 2007; Bodhini et al., 2007). This SNP is thought to modulate insulin secretion, resulting in the weakening of insulin discharge, and manipulating the activity of several other genes that govern glucose metabolism (Zeini et al., 2024).

The intricate pathways linking the TCF7L2 gene with an individual's hereditary risk of developing T2DM are still to be uncovered. TCF7L2, as described earlier, functions as a nuclear receptor in the Wnt signaling pathway and encodes for basic transcription factor 4, TCF-4. Of the several elements of the Wnt signaling pathway, B-catenin is the most important. Its participation is essential because once it binds to TCF-4, they constitute the B-catenin/TCF4 complex, which is the principal effector of the Wnt pathway. This complex is critical in the development of the pancreatic islets and also regulates the expression of several hormone genes (Mustafa and Younus et al., 2021).

This study intends to examine the relationship of TCF7L2 rs7903146 polymorphism with T2DM in Middle Euphrates population. Also, it aims to assess the effect of the polymorphism on major metabolic parameters such as fasting blood glucose, HbA1c, insulin, lipids, and HOMA-IR. Finding such associations will aid in identifying genetic predispositions and allow clinicians to more effectively intervene with tailored strategies guided by precision medicine principles.

METHODS

Study Population

A case-control study was carried out with 300 participants, divided into two equal groups, 150 for each: group of T2DM (Type 2 Diabetes Mellitus) and a healthy control group. The T2DM group consists of (68 males and 82 females) from the Diabetes Center in Al-Sadder Medical City, Najaf, Iraq. The diagnosis was done based on the criteria set by American Diabetes Association (ADA), which includes FPG (fasting plasma glucose) ≥126 mg/dL and/or HbA1c ≥6.5%, along with some clinical signs and symptoms. The subjects in the control group were of similar age with no family or personal medical history of diabetes or cardiovascular disease (American Diabetes Association et al., 2021). Standard protocols were followed for anthropometric measurements such as height and weight in order to calculate Body Mass Index (BMI) using the formula: weight (kg)/height (m^2). Ethical clearance was granted from the Faculty of Medicine, University of Kufa.

Biochemical Measurements

After an overnight fast, each participant had 5 mL of venous blood drawn. The sample was divided into three portions: A. a gel tube for serum separation B. an EDTA tube for DNA extraction, C. an EDTA tube for HbA1c assessment. Enzymatic measurement of fasting blood glucose was done using the glucose oxidase-peroxidase method (Pullano et al., 2022; Shaker & Swift, 2023). For assessing HbA1c, High-Performance Liquid Chromatography (HPLC) was performed using a GH-900Plus analyzer (Hu et al., 2021). The lipid profile included triglycerides (TG), total

cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C). These were all estimated with the use of enzymatic colorimetric methods. LDL-C and very low-density lipoprotein cholesterol (VLDL-C) were calculated with the use of the Friedewald equation (Friedewald et al., 1972). Serum insulin levels were analyzed with an sandwich ELISA kit, while the insulin resistance diagnosis was done using the Homeostasis Model Assessment of Insulin Resistance (HOMA-IR) method, using the following formula: HOMA-IR = [Glucose (mg/dL) × Insulin (μU/mL)] / 405 (Matthews et al., 1985).

DNA Extraction

Genomic DNA was extracted from whole blood samples using the Geneaid DNA mini kit. The quality and concentration of DNA were confirmed using a BioDrop spectrophotometer, assessing the A260/A280 ratio (ideal range 1.6–1.8) and the 260/230 ratio (2.0–2.2), to ensure purity (Lucena-Aguilar et al., 2016).

2.4 Genotyping of TCF7L2 rs7903146 SNP

Genotyping of the TCF7L2 rs7903146 polymorphism was carried out using the PCR-Tetra Amplification Refractory Mutation System (PCR-TETRA ARMS). Primer sets, consisting of two allele-specific inner primers (forward inner: TAGAGAGCTAAGCACTTTTTAGATAC, reverse inner primer CTCATACGGCAATTAAATTATATAA) and two outer primers (forward outer: AATTTTTTCACATGTGAAGACATAC, revers outer primer: TTTATAGCGAAGAGATGAAATGTAG) were designed based on previously described guidelines by (Mustafa and Younus et al., 2021). PCR reactions were achieved in 25 μl volumes comprising of 12.5 μl master mix (A GoTaq® G2 Green Master Mix, Promega , USA), 1 μl of each primer, a template DNA 6 μl, nuclease-free water up to 25 μl. The cycling conditions included an initial denaturation step at 95°C for 5 minutes, followed by 35 cycles of denaturation at 95°C for 45 seconds, annealing at an optimized temperature (54°C for 1minute), extension at 72°C for 1minute, and a final extension at 72°C for 10 minutes. PCR products were electrophoresed on 2% agarose gels, visualized under UV light, and documented.

Statistical Analysis

All analyses were performed using SPSS software version 25. Continuous variables were reported as mean ± standard deviation (SD) and were evaluated with Student's t-test. Biochemical differences between the genotypes were assessed with one-way ANOVA. Moreover, Bayesian techniques were added to the fundamental methods described above to enhance the previously mentioned primary techniques. Every statistical evaluation was conducted at a significance level of less than 0.05. Hardy-Weinberg equilibrium (HWE) was assessed by means of χ^2 testing to determine whether observed genotype frequencies diverged from expected proportions (Namipashaki et al., 2015). Moreover, Odds ratios (ORs) with 95% confidence intervals (CIs) were calculated from logistic regression analysis aimed to evaluate the impact of genetic parameters on the likelihood of developing diabetes.

RESULTS

In this study, we combined 300 subjects in two groups of 150 T2DM cases and a control group. The Bayesian and Independent Samples T-Test comparisons of the biochemical and anthropometric aspects between contributors reveal a clear and strong distinction in metabolic and biochemical markers between diabetic cases and healthy controls, and the results show the following as illustrated in Table (1). No significant

differences in age, body mass index (BMI) or gender has been recorded between the two groups ($p > 0.05$, $BF10 < 1$), suggesting that the two populations are comparable in terms of demographic parameters. Thus, these factors would not influence the differences in metabolism ascribed to diabetes.

On the contrary, all glycemic markers including (Fasting Blood Sugar and HbA1c) as well as HOMA-IR and components of the lipid profile (HDL, LDL, Triglycerides, Total Cholesterol, VLDL) demonstrated highly significant differences ($p < 0.001$) along with extremely high Bayes Factors (BF10) providing strong evidence of a true difference while insulin level indicate moderate difference ($p=0.01$) between two groups which suggest disease progression. Additionally, the Bayesian approach reinforced the results of the classical t-test, as indicated by the extreme BF10 values ($>10^{65}$ for FBS, $>10^{72}$ for HbA1c, and similarly high values for lipid markers) for the evidence of true differences between cases and controls. The Amplification of rs7903146 SNP Genotyping results were visualized through 2% agarose gel electrophoresis resulting in the production of three genotypes; CC, CT, and TT (Fig.1). The expected product sizes of the rs7903146 variant were 432 bp for the non-specific band, 211 bp for the C allele, and 272 bp for the T allele.

The genetic power for detecting an effect at rs7903146 was only 41%, which is considered insufficient, suggesting that the sample size or allele frequency might not have been optimal to reliably detect associations. Furthermore, there was a significant deviation from HWE ($p < 0.0001$) in the control group.

Genotype and allele frequencies in TCF7L2 gene polymorphism (rs7903146) show No significant association between genotypes and diabetes status under all genetic model (codominant, dominant, over-dominant, and recessive) in both crude and adjusted as illustrated in Table (2). Adjustment for age, gender, BMI did not importantly change the observed associations, showing no strong effect. Minor allele frequency (T allele) didn't consider a risk factor for diabetes in this population (p-value=0.151).

Table (1): Biochemical and clinical characteristics of study subjects.

parameters	Control subject	T2DM subject	P-value	BF10
No. (M/F)	150 (66/84)	150 (68/82)	0.5	
Age (year)	48.83 ± 8.29	49.99 ± 9.31	0.25	0.236
BMI (kg/m2)	27.22 ± 3.36	27.83 ± 3.24	0.11	0.426
FBS (mg/dl)	88.29 ± 6.06	181.92 ± 48.66	<0.001	$3.82*10^{65}$
Hb1AC	4.85 ±0.29	8.66 ± 1.82	< 0.001	$1.89*10^{72}$
Cholesterol(mg/dl)	163.98±15.48	218.18±36.25	<0.001	$3.97*10^{41}$
Triglyceride (mg/dl)	110.19 ± 16.65	196.04 ± 57.04	< 0.001	$6.02*10^{44}$
VLDL-C (mg/dl)	22.09 ± 3.37	39.21 ± 11.41	< 0.001	$3.34*10^{44}$
LDL-C (mg/dl)	93.76 ± 13.38	141.58 ± 33.80	<0.001	$7.84*10^{38}$
HDL-C (mg/dl)	48.74 ± 3.69	37.39 ± 7.71	<0.001	$2.79*10^{39}$
Insulin (µU/ml)	4.6 ± 6.4	7.1 ± 6.60	0.01	3.06
HOMA-IR	1.0 ± 1.4	3.2 ± 4.0	< 0.001	8067

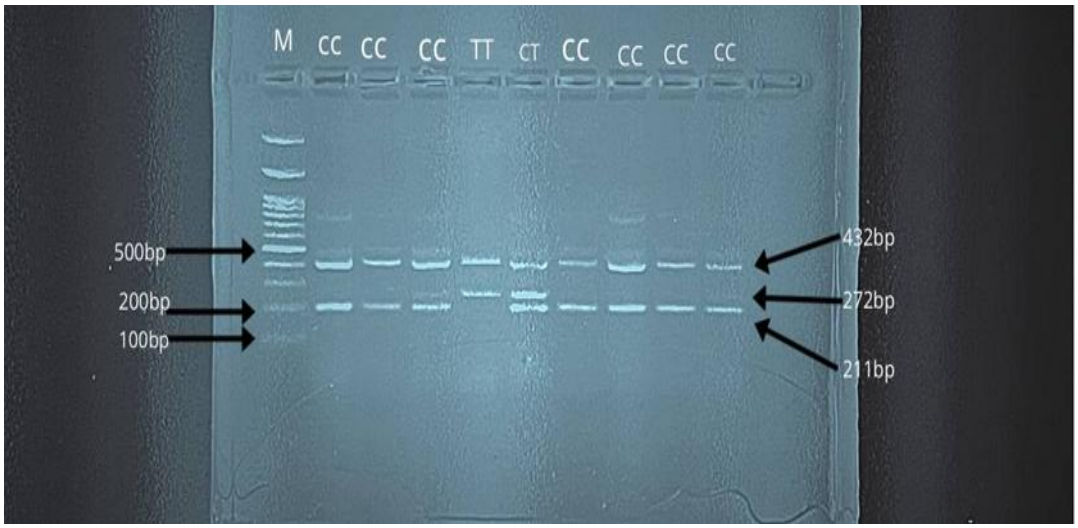

Figure 1: PCR-TETRA-ARM forTCF7L2 gene rs7903146 (c > T) polymorphism on Agarose gel electrophoresis (2%). M: molecular DNA marker 100 bp which has 12 bands ranging from 100-3000 kb, CC: homozygous genotype (wild type), CT: heterozygous genotype, TT: homozygous genotype (mutant type).

Table (2): Results of genotype and allele frequency of TCF7L2gene polymorphism (rs7903146) in patient and control groups in both crude and adjusted by age, gender, BMI.

Model	Genotype	control	T2DM	OR (95% CI)	P-value	Adjusted OR (95% CI)	P-value
Codominant	C/C	141 (94%)	135 (90%)		0.42		
	C/T	6 (4%)	9 (6%)	1.57 (0.54-4.52)		1.35 (0.45-4.02)	0.63
	T/T	3 (2%)	6 (4%)	2.09 (0.51-8.52)		1.82 (0.43-7.70)	0.63
Dominant	C/C	141 (94%)	135 (90%)		0.2		
	C/T-T/T	9 (6%)	15 (10%)	1.74 (0.74-4.11)		1.50 (0.62-3.66)	0.37
Recessive	C/C-C/T	147 (98%)	144 (96%)		0.31		
	T/T	3 (2%)	6 (4%)	2.04 (0.50-8.32)		1.78 (0.42-7.53)	0.42
Over dominant	C/C-T/T	144 (96%)	141 (94%)		0.43		
	C/T	6 (4%)	9 (6%)	1.53 (0.53-4.42)		1.31 (0.44-3.91)	0.62
MAF	T	0.04	0.07	1.81 0.87, 3.74)	0.151		

DISCUSSION

This study explored the association of the TCF7L2 rs7903146 (C>T) polymorphism with Type 2 Diabetes Mellitus (T2DM) susceptibility in the Middle Euphrates population of Iraq. Contrary to findings from several other populations, we observed no significant association between the rs7903146 variant and T2DM risk under any genetic model (codominant, dominant, recessive, or over-dominant), even after adjusting for potential confounders such as age, gender, and BMI. Furthermore, no significant impact of this SNP on metabolic traits, including fasting blood glucose, HbA1c, lipid profile, insulin levels, or insulin resistance (HOMA-IR), was detected among different genotypic groups.

These results are consistent with previous studies conducted in other Middle Eastern populations. For instance, similar null associations were reported in Iraqi populations (Kaftan, 2015; Mustafa & Younus, 2021), as well as among Emirati (Saadi et al., 2008) and Saudi cohorts (Alsmadi et al., 2008). In contrast, substantial associations between rs7903146 and T2DM risk have been established in European populations (Grant et al., 2006; Cauchi et al., 2007), as well as certain Asian cohorts (Bodhini et al., 2007; Hayashi et al., 2007), where the T allele was shown to impair β-cell function and elevate fasting glucose levels.

The discrepancy between our findings and those of other populations suggests a potential ethnic-specific effect. Factors such as differences in linkage disequilibrium patterns, gene-environment interactions (e.g., diet, physical activity), and genetic heterogeneity likely contribute to the observed variation in the impact of rs7903146 across populations (Wray, 2008). For instance, the Middle Euphrates population might possess distinct environmental exposures or genetic backgrounds that modulate the effect of TCF7L2 on glucose metabolism.

Another key consideration is the statistical power of this study. The power to detect an effect at rs7903146 was only 41%, which is below the recommended threshold for genetic association studies (typically ≥80%) (Menashe et al., 2008). This limited power likely stems from the relatively low minor allele frequency (MAF) of the T allele (0.04 in controls) and the moderate sample size. Consequently, while no association was detected, the possibility of a modest genetic effect cannot be entirely excluded. Future studies with larger sample sizes and more robust power calculations are necessary to clarify these findings.

Additionally, the observed deviation from Hardy-Weinberg Equilibrium (HWE) in the control group suggests potential issues such as population stratification, selection bias, or genotyping errors (Zintzaras, 2010). Although genotyping was conducted using validated PCR-Tetra ARMS protocols, further validation with alternative genotyping methods or replication in independent cohorts would strengthen the reliability of these results.

Lastly, the lack of association between rs7903146 genotypes and metabolic parameters (e.g., insulin resistance, lipid profiles) in this study further supports the conclusion that this SNP does not play a major role in T2DM pathogenesis in this population. These findings emphasize the need to consider population-specific genetic and environmental factors when investigating the genetic architecture of complex diseases like T2DM.

To address these issues, future studies should aim to increase sample sizes to improve statistical power and include participants from diverse Iraqi and regional subpopulations to enhance generalizability. Additionally, replication of the findings using alternative genotyping methods, such as TaqMan assays or sequencing, would provide further validation. Incorporating environmental and lifestyle data, such as dietary habits and physical activity levels, would also allow for the assessment of potential gene-environment interactions that may modulate genetic risk. Furthermore, the application of genome-wide association studies (GWAS) and polygenic risk scores could provide a more comprehensive understanding of the genetic architecture underlying T2DM in the Iraqi population.

CONCLUSION

This research does not show any important links between the TCF7L2 rs7903146 polymorphism and the likelihood of developing Type 2 Diabetes Mellitus in people from the Middle Euphrates region of Iraq. To fully understand the impact that rs7903146 might have on the susceptibility to diabetes, more thorough studies that include consideration of environmental factors are essential.

Ethical approval

The present study Which is conducted by (ANK, HAA) was approved by the local Department of Biochemistry and Kufa ethical committee.

Statement of Permission and Conflict of Interests

The authors declare that they have no competing interests.

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