Biomedical & Pharmacology Journal, December 2022. Vol. **15**(4), p. 2403-2407

Association of Transcription Factor 7 Like 2 (TCF7L2) (rs7903146) Gene Polymorphisms with Some Trace Elements in the Type 2 Diabetes Mellitus Patients in Al-Najaf Governorate

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https://dx.doi.org/10.13005/bpj/2578

(Received: 24 June 2022; accepted: 21 September 2022)

The widespread prevalence of diabetes has led to a serious health risk for humans. Hyperglycemia is a hallmark of diabetes. Assessing the relation of TCF7L2 gene polymorphism (rs7903146) on the occurrence of T2DM in the Alnajaf population, and evaluating the impact of this investigated SNP on phenotypic parameters like BMI, fasting blood sugar(F.B.S), lipid profile , Mg and Zn. A case-control study of 800 individuals (400 T2DM and 400 apparently healthy control individuals) was done. The laboratory work was carried out in the laboratory of Department of Clinical Laboratory Sciences in the Faculty of Pharmacy and in the laboratory of Department of Chemistry in the Faculty of Sciences / the University of Kufa. DNA from whole blood was extracted and genotyping of TCF7L2 gene (rs7903146) polymorphism polymorphisms were carried out by RFLP –PCR. The results of this study showed that there is a significant association between the transcription factor 7-like 2 (TCF7L2) gene polymorphisms rs7903146 gene polymorphism with T2DM in the Alnajaf population. TCF7L2 gene polymorphism (rs7903146) did not show any major changes in relation to BMI, FSG, cholesterol, HDL-C, VLDL-C, or T.G, but there were significant changes in LDL, magnesium, and zinc.

Keywords: Al-Najaf; Diabetes Mellitus; type 2; Polymorphism; Transcription Factor;TCF7L2.

Chronic hyperglycemia due to changes in insulin secretion, insulin action, or both characterizes the metabolic diseases known collectively as diabetes mellitus. Insulin's role as an anabolic hormone contributes to metabolic abnormalities in carbs, fats, and proteins [1]. These metabolic disorders are caused by insulin resistance and/or low levels of insulin at the receptor, signal transduction system, or effector enzyme or gene levels in target tissues, notably skeletal muscles and adipose tissue, and to a lesser extent liver [2]. Approximately 415 million adults worldwide have diabetes, and nearly half of them (46.5%) have never been diagnosed. Every six seconds, someone dies from diabetes (5.0 million deaths per year) [3]. About 642 million people in the United States will have diabetes by the year 2040. The majority of the $673 billion spent annually on healthcare around the world goes toward diabetes care (12 percent) [4].

The TCF7L2 gene has received a lot of attention because of the strong correlation between the two (T2DM) [5]. Originally connected with

development biology, the Wnt signaling pathway is a complex network of interacting proteins that regulates cellular intercommunications at multiple levels and has a wide variety of consequences. The TCF7L2 gene is a key component of this route [6]. TCF7L2 is just one example of a Wnt network-associated protein that has been related to a wide range of diseases and cancer models, demonstrating the importance of this developmental pathway in the pathogenesis of human disorders [7].

The TCF7L2 gene's frequent polymorphism, rs7903146(C/T), is linked to an increased risk of type 2 diabetes if you have the riskier T allele [8]. The TCF7L2 gene product, a high mobility group box-containing transcription factor previously related in blood glucose management, is assumed to be involved in Wnt signaling's regulation of proglucagon gene expression in enteroendocrine cells [9]. There is a positive correlation between TCF7L2 expression and insulin gene expression in human islets [10].

Consequently, we set out to investigate the associations between polymorphisms in the Transcription factor 7 like 2 gene (TCF7L2) and type 2 diabetes, as well as the effects of this association on biochemical and clinical outcomes.

MATERIALS AND METHODS

Collection of Samples

Four hundred people with type 2 diabetes participated in the study (200 male and 200 female). The ages of our patients ranged from 40 to 70, with a mean sd of 52.93 7.00. Four hundred participants who were otherwise healthy were assigned to the "healthy" group (200 male and 200 female). AL-Sadr Medical City in al Najaf Province is where they were selected from

PCR Assay

The Intron kit makes blood DNA extraction and purification much easier. High-purity strands of DNA have been successfully employed in agarose gel electrophoresis, restriction enzyme digestion, and polymerase chain reaction (PCR). There are four fundamental stages to this procedure. Methods for Isolating Genomic Information from Frozen Blood Samples Consistent with the instructions provided in the Intron Kit (iNtRoN, Biotech. Inc., Korea). As part of the gel documentation procedure (Cleaver, United Kingdom), 1% agarose (iNtRoN, Biotech, Inc., Korea) was used to examine and disperse the migration of PCR bands, and the gel was back-dyed with ethidium bromide at a concentration of 0.5 g/ml. These studies employ RFLP-PCR, and the primer sequence and restriction enzyme employed in the experiment are listed in the table below.

Statistical Analysis

The SPSS V.25 program was used in statistical analysis of the data. To analyze the disparities between these factors, the chi-square test was applied. Continuous parameters' means were compared among genotypes using the ANOVA test.

RESULTS AND DISCUSSION

Genetic Polymorphism of TCF7L2 rs7903146

The PCR products of TCF7L2 gene SNP (rs79103146) are 175bp

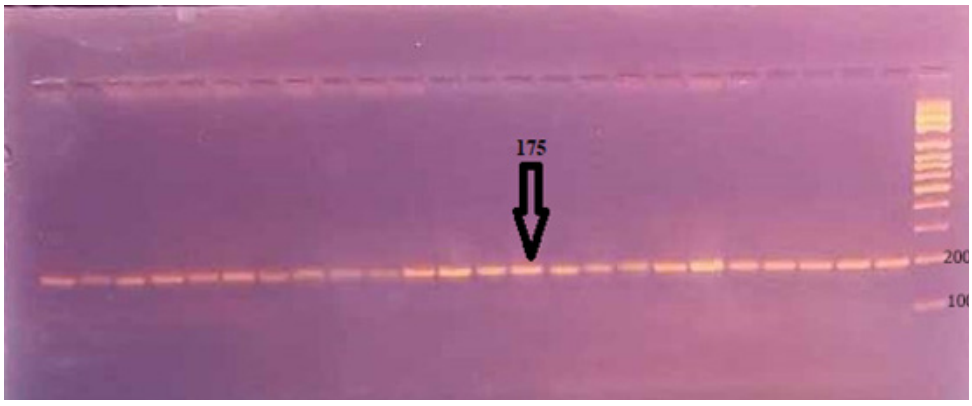

Fig. 1. PCR product of TCF7L2 gene polymorphism (rs79103146) analyzed by agarose gel electrophoresis

RFLP analysis for TCF7L2 gene

Restriction enzymes were used for digestion of TCF7L2 amplicon the resulted products were subjected to electrophoresis as in the following:

There were no significant differences in the distributions of TCF7L2 genotypes when comparing body mass index (B.M.I), fasting glucose, cholesterol, high-density lipoprotein cholesterol, very low-density lipoprotein cholesterol, or total cholesterol to triglyceride levels or triglyceride levels with [12] but there were significant changes in LDL, magnesium, and zinc.

Variations in body mass index (BMI), cholesterol, high-density lipoprotein (HDL), low-

Table 1.

Gene	Primer	Sequence	T_m(ºC)
TCF7L2 rs7903146	Forward	52 -TTA GAG AGC TAA GCA CTT TTT AGG TA-32	61.6
	Reverse	52 -AGA GAT GAA ATG TAG CAG TGA AGT G-32	62.5

Table 2. Results for digested (rs7903146) polymorphism of TCF7L2 gene

Genotyping		Bands number	Size
Wild	CC	2	20 and 80
Hetero	CT	3	20,80, and 175
Homo	TT	1	175

Table 3. Genotyping and frequency of alleles of TCF7L2(rs7903146) polymorphism in studied group

C/T	Control (n= 400)	T2DM (n= 400)	Unadjusted OR (95% CI) P-Value	adjusted OR (95% CI) P-Value
Codominant				
CC	260	185	REF	REE
CT	115	172	2.1 1.55-2.84 P < 0.0001	3.13 2.05-4.76 P < 0.0001
TT	25	43	2.42 1.43 - 4.10 0.001	2.57 1.51-4.38 P < 0.0001
Dominate				
CT+ TT	140	215	2.16 1.62- 2.87 P < 0.0001	2.34 1.75-3.12 P < 0.0001
Recessive				
CC + CT(REF)	375	357	———	———
TT	25	43	1.81 1.08 - 3.02 0.024	1.87 1.12-3.15 0.018
Additive				
2(TT)+CT	165	258	2.31 1.76 to 3.04 P < 0.0001	2.83 1.95-4.12 P < 0.0001
Frequency of T allele %	20.60%	31%	2.1 1.50 - 2.94 P < 0.0001	2.26 1.61-3.18 P < 0.0001

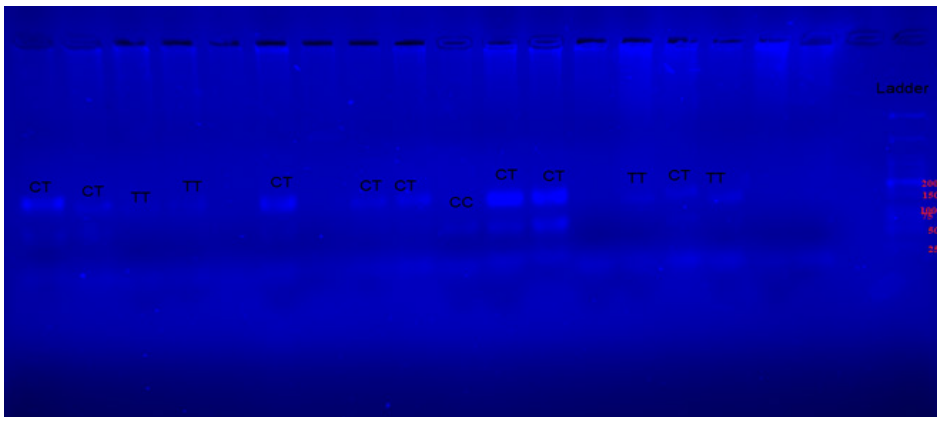

Fig. 2. Digested products in agarose electrophoresis for TCF7L2 gene (rs7903146) polymorphism

Table 4. The relation between TCF7L2 (rs7903146 C>T) gene polymorphism genotypes and the investigated parameters in patients' group under the codominant model

Clinical Characteristic	Genotype M ± SD			P value	
	CC(n=185)	CT (n=172)	TT(n=43)	CC CT CC TT	CT TT
BMI	29.34 ±5.08	28.41 ± 5.15	28.10±5.09	0.089 0.724	0.154
FPG (mg/dl)	181.58 ± 63.59	184.99 ± 78.28	194.47 ± 93.89	0.663 0.303	0.452
Cholesterol (mg/dl)	269.17 ± 26.90	269.05 ± 26.94	270.05 ± 27.25	0.965 0.848	0.828
HDL-C (mg/dl)	37.71± 6.45	36.88 ± 7.21	36.74± 7.32	0.251 0.402	0.905
Triglycerides(mg/dl)	242.84 ± 47.70	247.98 ± 48.70	247.01 ± 47.97	0.314 0.609	0.906
VLDL (mg/dl)	48.57± 9.54	49.6 ± 9.74	49.4 ± 9.59	0.314 0.609	0.906
LDL (mg/dl)	162.39 ± 34.76	319.0 ± 173.18	311.10 ± 171.51	< 0.0001 < 0.0001	0.719
Mg (mg/dl)	1.4588 ± 0.33551	1.2107 ± 0.36692	1.1999 ± 0.35733	< 0.0001 < 0.0001	0.857
Zn (mg/dl)	81.05 ± 25.77	58.16± 26.70	50.60 ± 28.16	< 0.0001 < 0.0001 < 0.0001 0.094	0.094

Table 5. The relation between TCF7L2 (rs7903146 C>T) gene polymorphism genotypes and the investigated parameters in patients' group under the dominant model

Clinical Characteristic	Genotype M ± SD		
	CC(n=185)	CT + TT(n=215)	p-value
BMI	28.18 ± 5.0	29.24 ± 5.0	0.037
FSG (mg/dl)	181.56 ± 63.59	183.93 ± 77.22	0.743
Cholesterol(mg/dl)	248.67 ± 33.32	403.83 ± 172.16	< 0.0001
HDL (mg/dl)	37.71 ± 6.46	36.85 ± 7.22	0.0210
Triglycerides(mg/dl)	242.84 ± 47.7	247.78± 48.4	0.306
VLDL (mg/dl)	48.57 ± 9.5	49.5 ± 9.7	0.306
LDL (mg/dl)	162.4 ± 34.76	317.42 ± 172.47	< 0.0001
Mg (mg/dl)	1.45 ± 0.33	1.2 ± 0.36	< 0.0001
Zn (mg/dl)	53.94±24.43	79.83± 27.47	< 0.0001

density lipoprotein (LDL), magnesium, and zinc all show substantial differences when compared to the dominant genotype distribution.

Low insulin levels, reduced waist circumference, and better lipid profiles were associated with the TCF7L2 rs7903146 polymorphism in the general senior population of Tuscany, Italy. Patients with diabetes who carry the minor allele are at reduced risk for developing metabolic syndrome [13].

CONCLUSIONS

TCF7L2 gene (rs7903146) polymorphism, was correlated with the tendency to T2DM in Alnajaf populations. TCF7L2 gene polymorphism (rs7903146) did not show any major changes in relation to BMI, FSG, cholesterol, HDL, VLDL, or T.G, but there were significant changes in LDL, magnesium, and zinc.

Recommendations

1- Study other SNPs of TCF7L2 gene to assess their association with T2DM.

2- Sequencing of the examined gene in order to find new SNPs associated with T2DM.

3- Haplotype study of the gene investigated to determine their association with type 2 diabetes.

5- Analysis of more SNPS of TCF7L2 gene to determine which one is more common in our population.

6- Evaluation of gene expression to determine the effect of SNPS on the various phenotypic properties.

7- DNA sequencing may give more effective results in regard to the correlation of SNPS on the various phenotypic parameters in type 2 diabetic patients.

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