Archives of Razi Institute, Vol. 76, No. 4 (2021) 841-846

DOI: 10.22092/ari.2021.355851.1729

Original Article

Sequence Analysis of Transcription Factor 7-like 2 Protein (TCF7L2) in Iraqi Patients with Diabetic Mellitus Type 2 Using Bioinformatics Methods

Kareem Queen, B[1 *], Abdullah Al-Janabi, A[1]

1. Department of Applied Sciences, University of Technology, Baghdad, Iraq

Received 27 August 2021; Accepted 14 September 2021
Corresponding Author: batoolkareem97@gmail.com

Abstract

Several previously published reports have suggested a relationship between type 2 diabetes mellitus (T2DM) and chromosome 10q. The results of genotyping of 228 microsatellite markers in Icelandic people with T2DMrevealed that a microsatellite, DG10S478, within intron 3 of the transcription factor 7-like 2 gene (TCF7L2; formerly TCF4) was associated with T2DM. The present study was aimed to analyze the sequence of TCF7L2 in Iraqi patients with T2DM. This study was performed on the blood samples of 10 patients within the age range of 18-70 years old with T2DM. The DNA was extracted from the whole blood samples and the TCF7L2 gene was purified and amplified using the polymerase chain reaction (PCR) technique. Afterward, the PCR products were run in gel electrophoresis to detect the gene. Moreover, the BLAST software was used to analyze the gene TCF7L2 sequence which was compared with the reference sequence of the template gene from NCBI. The results of TCF7L2 gene sequences obtained from the samples collected from the Iraqi patients with T2DMwere received from Macogen Company, Korea, and analyzed using the BLAST software. The findings showed mutations in the gene sequence of all patients, compared to the gene sequences in NCBI. Hence, the mutation in the TCF7L2 gene was present in Iraqi patients with T2DM, and it could be one of the factors causing and increasing the risk of T2DM disease.

Keywords: Bioinformatics Methods, Diabetic mellitus type 2, Mutation, TCF7L2 gene

1. Introduction

Bioinformatics is defined as the junction between molecular biology and computer science. This means that the emerging disciplines of biotechnology start from the application of mathematics, statistics, and information technology, including computer systems and the theories surrounding them, to store, study, and analyze macromolecular data sets (1, 2). Human Transcription Factor 7-like 2 Protein gene (TCF7L2) is sited on chromosome 10q25.2–q25.3, contains 19 exons, and encodes a high mobility group box containing transcription factor (3). The TCF7L2 protein has been embroiled with blood glucose homeostasis. Genetic variants of this gene have a direct relationship with the increased risk of type 2 diabetes (T2DM).In a previously published research, it was approved that several variations encoded by different isoforms, which were found in TCF7L2, are the key factors in the incidence of T2DM (4).

This kind of diabetes, which affects 90-95% of people and was previously known as non-insulin-dependent diabetes, T2DM, or adult-onset diabetes, is characterized by insulin resistance and relative (rather than absolute) insulin shortage. These patients do not require insulin treatment at first. However, the longer someone has T2DM, the more likely they are to require

insulin (5). The T2DM is a serious and common chronic disease resulting from a complex inheritance-environment interaction along with other risk factors, such as obesity and a sedentary lifestyle.

The T2DM and its complications constitute a major worldwide public health problem, affecting almost all populations in both developed and developing countries with high rates of diabetes-related morbidity and mortality. The prevalence of T2DM has been increasing exponentially, and it has a high prevalence rate in developing countries and also in populations undergoing "westernization" or modernization. Multiple risk factors of diabetes, delayed diagnosis until micro- and macro-vascular complications, life-threatening complications, failure of the current treatments, and financial costs for the treatment of this disease, make it necessary to develop new efficient treatment strategies and appropriate prevention measures for the control of T2DM. The majority of people with this kind of diabetes are obese, and obesity induces insulin resistance (6). In general, researchers believe that several genetic and environmental factors can lead to the incidence of T2DM.This study aimed to analyze the sequence of TCF7L2 gene in Iraqi diabetic patients using the bioinformatics method.

2. Material and Methods

The study was performed on10 Iraqi patients with T2DM within the age range of 18-70 years old. The samples were collected from patients in Al-Mustansiriya University National Diabetes Center in Baghdad province, Iraq. The collected blood samples were stored at -20° C until use (7).

The pure DNA of the TCF7L2 gene was extracted from whole blood samples using Quick-gDNA™ Blood Mini DNA extraction kit (Cat.No.: D3024& D3025,Zymo, USA) according to the protocols of the manufacturer. Agarose gel electrophoresis was used to confirm the presence of the extracted DNA.

The TCF7L2 gene sequences were taken from National Center for Biotechnology Information (NCBI) database. Moreover, the primers were designed for the TCF7L2 gene using two primers provided by an integrated DNA technologies company, Canada. The sequences of both forward and reverse primers are summarized in table 1.

The TCF7L2 primers were used to amplify the TCF7L2 gene by polymerase chain reaction (PCR) pre-mix kit (Intron, Korea) according to the instructions of the manufacturer. The mixture of PCR solution is tabulated in table 2.

The condition of PCR reaction to amplify the TCF7L2 gene is summarized in table 3.

The gel electrophoresis was performed using 2% agarose gel, and the gel was visualized using UV-light after staining it with Red Safe Nucleic acid stain (Intron, Korea) which was bound to DNA and emitted fluorescence at a wavelength of 537nm.

Amplified PCR products of the TCF7L2 gene for 10 patients with T2DM were sent to Macrogen Company in Korea for sequencing the TCF7L2 gene. Moreover, they were compared with the reference sequence of the TCF7L2 gene in NCBI using Basic Local Alignment Search Tool (BLAST) software (8) to detect the variations in the gene sequence.

Table 1. Sequences of both forward and reverse primers were used for TCF7L2 gene amplification.

Primer	Sequence	Tm(°C)	GC (%)	Product size
Forward	5'- TCATAGGGGTCTGGCTTGGA- 3'	57.8	55	888 bp
Reverse	5'-TGATGAACGGGGACTTGCTC - 3'	57.3	55	

Table 2. Components of polymerase chain reaction solution for amplification of TCF7L2 gene.

Components	Concentration
Taq PCR Pre Mix	5µl
Forward primer	5 picomols/µl (1µl)
Reverse primer	5 picomols/µl (1µl)
DNA	1.5 µl
Distill water	16.5 µl
Final volume	25µl

Table 3. The optimum conditions of the detection of TCF7L2 gene.

No.	Phase	Tm(°C)	Time	No. of cycle
1-	Initial Denaturation	95°C	3 min	1cycle
2-	Denaturation -2	95°C	45sec	35 cycles
3-	Annealing	64°C	45sec	
4-	Extension-1	72°C	45sec	
5-	Extension -2	72°C	7min	1 cycle

3. Results and Discussion

3.1. Detection of TCF7L2 gene by PCR Technique

The results were shown using two primers that were used to amplify the TCF7L2 gene exon as a specific primer. The result of amplification segments of DNA of T2DM patients by primers lead to pure PCR bands with the size of 888 bp after electrophoresis on 2% agarose gel at 75 volts for 60 min as shown in figure 1.

3.2. Sequence Alignment of TCF7L2 gene

The results of TCF7L2 gene sequences for 10 patients with T2DM were received from Macrogen Company, Korea, and then analyzed using BLAST software. The results showed a mutation in the gene sequence of all patients, compared to the sequence of the gene in NCBI.

In the first patient sample, mutations appeared in thymine transversion to adenine (T to A) at both sites of 104074 and 104288, thymine transition to cytosine (T to C) at site 104280, cytosine transversion to thymine (C to T) at site 104283 and adenine (C to A) at site 104286.

In the second patient sample, mutation appeared in guanine transversion to thymine (G to T) at site 103894, thymine transversion to adenine (T to A) at both sites 104074 and 104505, and 18-thymine transversion to adenine (T to A) at sites 104074 and 104505.

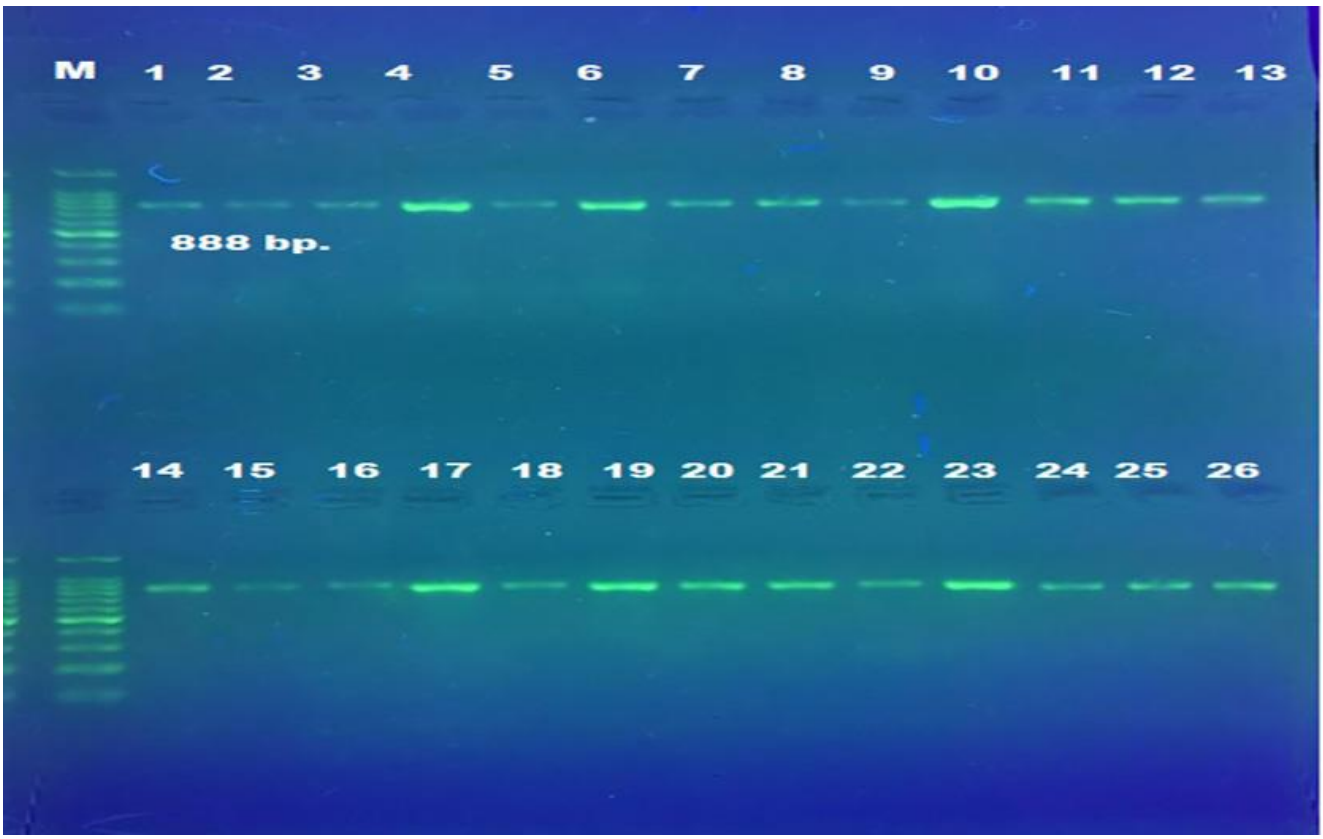

Figure 1. Products of polymerase chain reaction gel electrophoresis on 2% agarose showed clear bands with the size of 888bp of TCF7L2 gene in Iraqi patients with type 2 diabetes mellitus.

In the third patient sample, mutation appeared in guanine transversion to thymine (G to T) at site 103894 and thymine transversion to adenine (T to A) at site 104505.

In the fourth patient sample, a mutation appeared in thymine transversion to adenine (T to A) at site 104074 and guanine transition to adenine (G to A) at site 104533.

In the fifth patient sample, a mutation appeared in thymine transversion to adenine (T to A) at sites 104074 and 104505 and guanine transition to adenine (G to A) at both sites 104533 and104621.

In the sixth patient sample, a mutation appeared in transversion of thymine to adenine (T to A) to sites 104074 and104505, 23- transversion of thymine to adenine (T to A) at site 104505.

In the seventh patient sample, a mutation appeared in transversion of adenine to thymine (A to T) at sites 103996 and 104201, thymine to guanine (T to G) at site 104037, adenine transition to guanine (A to G) at site 104090, cytosine transversion to Guanine (C to G) at site 104194, and transversion of thymine to adenine (T to A) at site 104506.

In the eighth patient sample, a mutation appeared in transversion of thymine to adenine (T to A) at sites 104074 and 104275, cytosine to adenine (C to A) at site 104277, adenine transition to guanine (A to G) at site 104278, transversion of guanine to cytosine (G to C) at site 104279, and transition of cytosine to thymine (C to T) at site 104283.

In the ninth patient sample, mutation appeared in transversion of thymine to adenine (T to A) at sites

104064,104074, and 104505 as well as adenine to thymine (A to T) at site 103996.

In the tenth patient sample, mutation appeared in transversion of thymine to adenine (T to A) at sites 104064,104074, and 104505 as well as adenine to thymine (A to T) at site 103996.

In all patients with T2DM, transversion mutation appeared in the sequence of TCF7L2 gene when the adenine wasinserted instead of thymine in the sites 104074 and 104288 as shown in figure 2. It shows the insertion of Adenine (A) in the sites 104074 and 104288 in the sequence of TCF7L2 gene for a patient with T2DM, compared to the sequence of TCF7L2 gene in the NCBI as a part of the results by BLAST software.

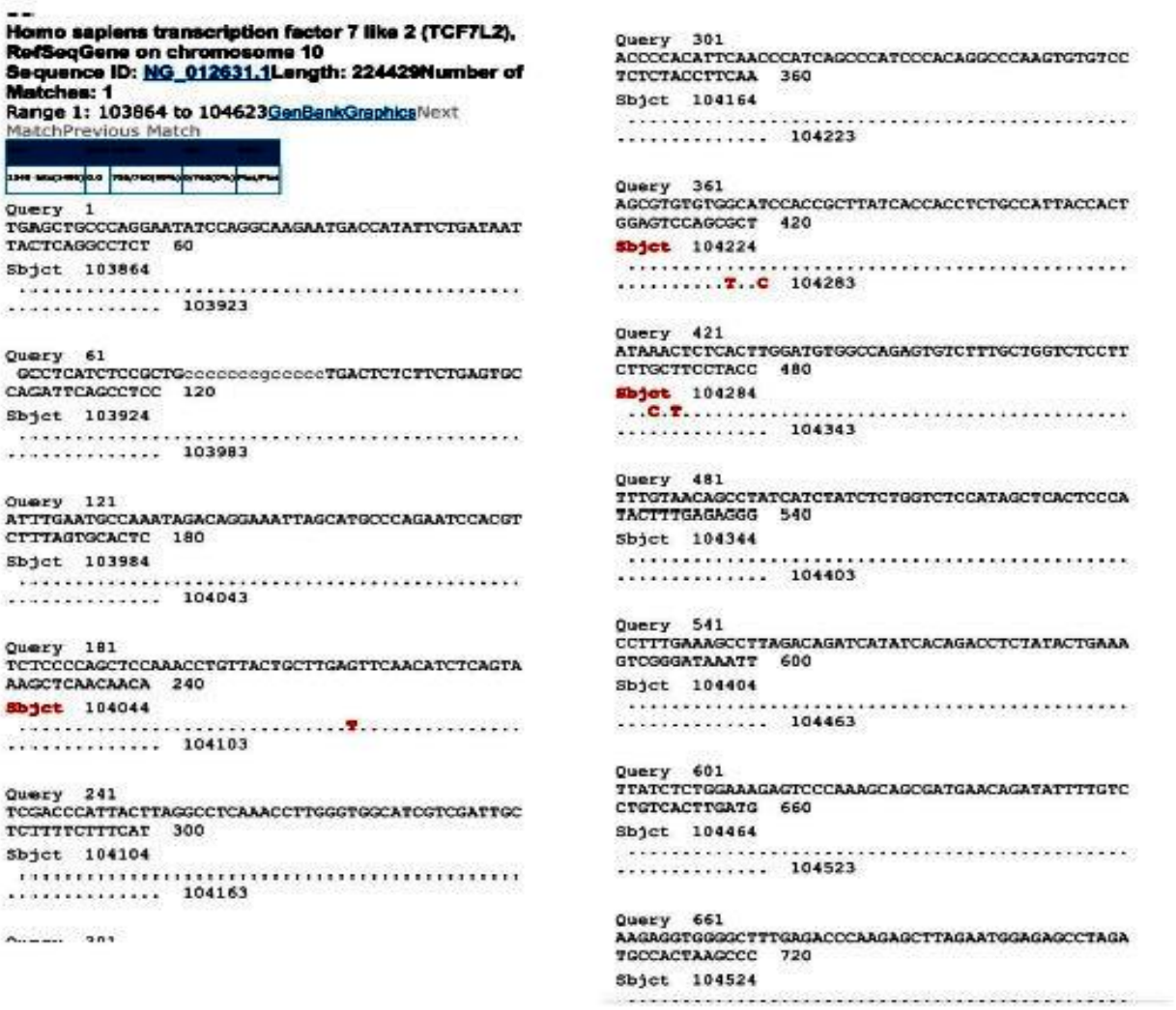

Figure 2. Results of alignment between the sequence of TCF7L2 gene for T2DM patient (query) and the sequence of TCF7L2 gene in National Center for Biotechnology Information (sbjct) by BLAST software, where the mutation appeared between the sites 104074 and 104288 (indicated by the blue arrow).

Hyperglycemia is the main indicator ofT2DM and can occur via mechanisms, such as defeated insulin secretion and insulin resistance and also in enhanced glucose output by the liver. The results of the present study confirmed the importance of mutations of the TCF7L2 gene in the prevalence of T2DMin Iraqi patients.

The mutation in the TCF7L2 gene in Iraqi T2DM patients appeared in all samples which indicated that the mutations cause T2DM and increase the risk of T2DM. Moreover, it revealed that the TCF7L2 gene product is a high mobility group box containing transcription factors previously implicated in blood glucose homeostasis. These results were in line with those of other studies which showed that the mutation in the TCF7L2 gene in people of other countries led to a higher risk of T2DM (9, 10, 11). Moreover, the results were consistent with those of another study conducted by Grant et al. (10) demonstrating that a mutation in the TCF7L2 gene caused T2DM and increased the risk of this disease (12). In conclusion, the mutation in the TCF7L2 gene in Iraqi patients with T2DM causes and increases the risk of T2DM disease.

Authors' Contribution

Study concept and design: B. K. Q.
Acquisition of data: A. A. A.
Analysis and interpretation of data: B. K. Q.
Drafting of the manuscript: B. K. Q.
Critical revision of the manuscript for important intellectual content: A. A. A.
Statistical analysis: B. K. Q.
Administrative, technical, and material support: A. A. A.

Ethics

All procedures performed in the study involving human participants were in accordance with the ethical standards of the University of Technology, Baghdad, Iraq, under the project number of 2021-252587-54.

Conflict of Interest

The authors declare that they have no conflict of interest.

References

1. Cao Y, Geddes TA, Yang JYH, Yang P. Ensemble deep learning in bioinformatics. Nat Mach Intell. 2020;2(9):500-8.
2. Diniz W, Canduri F. Bioinformatics: an overview and its applications. Genet Mol Res. 2017;16(1).
3. NCBI. TCF7L2 transcription factor 7 like 2 [Homo sapiens (human)] - Gene 2017 [2017-11-30]. Available from: www.ncbi.nlm.nih.gov.
4. Ali O. Genetics of type 2 diabetes. World J Diabetes. 2013;4(4):114.
5. WHO. Diabetes Fact sheet N°312. 2012 2012-01-09. Report No.
6. Deshpande AD, Harris-Hayes M, Schootman M. Epidemiology of diabetes and diabetes-related complications. Phys Ther. 2008;88(11):1254-64.
7. Zhao Z, Li X, Jian D, Hao P, Rao L, Li M. Hsa_circ_0054633 in peripheral blood can be used as a diagnostic biomarker of pre-diabetes and type 2 diabetes mellitus. Acta Diabetol. 2017;54(3):237-45.
8. Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. Basic local alignment search tool. J Mol Biol. 1990;215(3):403-10.
9. del Bosque-Plata L, Martínez-Martínez E, Espinoza-Camacho MÁ, Gragnoli C. The Role of TCF7L2 in Type 2 Diabetes. Diabetes. 2021.
10. Grant SF, Thorleifsson G, Reynisdottir I, Benediktsson R, Manolescu A, Sainz J, et al. Variant of transcription factor 7-like 2 (TCF7L2) gene confers risk of type 2 diabetes. Nat Genet. 2006;38(3):320-3.
11. Kamel MD, Mohammed AA, Ibrahim AA. Sequence and Structure Analysis of CRP of Lung and Breast Cancer using Bioinformatics Tools and Techniques. Biosci Biotechnol Res Asia. 2018;11(1):163-74.
12. Huang Z-q, Liao Y-q, Huang R-z, Chen J-p, Sun H-l. Possible role of TCF7L2 in the pathogenesis of type 2 diabetes mellitus. Biotechnol Biotechnol Equip. 2018;32(4):830-4.