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

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Abstract

Previously several published report had been suggested the linkage of type 2 diabetes mellitus to chromosome 10q. The results of genotyping of 228 microsatellite markers in Icelandic individuals with type 2 diabetes revealed that a microsatellite, DG10S478, within intron 3 of the transcription factor 7-like 2 gene (TCF7L2; formerly TCF4) was associated with type 2 diabetes. The aim of the current study was to analysis sequence of TCF7L2 in Iraqi Patients with Diabetic mellitus type II. In the current study blood samples from 10 patients at the age of 18-70 year-olds with T2DM were obtained. The DNA was extracted from the whole blood of samples and the TCF7L2 gene was purifying and amplified using polymerase chain reaction (PCR) technique, then the PCR products were runned in gel electrophoresis which was used to detect the gene, The BLAST software used to analyzing the gene TCF7L2 sequence which compared with the reference sequence of the template gene from NCBI. The results of TCF7L2 gene sequences obtained from the samples obtained from the Iraqi patients with diabetic mellitus type II were received from Macogen Company/Korea, then analyzed using BLAST software, where the results showed mutations in the sequence of gene for all patients as compared to the sequence of gene in NCBI. The mutation in TCF7L2 gene is present in Iraqi diabetic mellitus type II patients. It could be one of the causing and increasing the risk of T2DM disease.

Keywords: Bioinformatics Methods, Diabetic mellitus type II, Mutation, TCF7L2 gene
1. Introduction

Bioinformatics defined as the junction between molecular biology and computer science that mean the emerging disciplines of biotechnology starting from the application of mathematics, statistics, and information technology, including computer systems and the theory surrounding them, to store, study and analysis 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 encodes a high mobility group (HMG) box-containing transcription factor (3). The TCF7L2 protein has been embroiled with blood glucose homeostasis. Genetic variants of this gene has direct relationship with the increased risk of type II diabetes. In the previous published research it was approved that several variations encoding by different isoforms of which founded in TCF7L2 are the key factors in the incidence of Type II diabetes (4). This kind of diabetes, which affects 90–95 percent of people and was previously known as non-insulin dependent diabetes, type II diabetes, or adult-onset diabetes, is characterized by insulin resistance and relative (rather than absolute) insulin shortage. These people do not require insulin treatment at first. However, the longer someone has type 2 diabetes, the more likely they will require insulin (5). Type 2 diabetes is a serious and common chronic disease resulting from a complex inheritance-environment interaction along with other risk factors such as obesity and sedentary lifestyle. Type 2 diabetes 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 type 2 diabetes has been increasing exponentially, and a high prevalence rate has been observed in developing countries and in populations undergoing “westernization” or modernization. Multiple risk factors of diabetes, delayed diagnosis until micro- and macro-vascular complications arise, life-threatening complications, failure of the current therapies, and financial costs for the treatment of this disease, make it necessary to develop new efficient therapy strategies and appropriate prevention measures for the control of type 2 diabetes. The majority of
people with this kind of diabetes are obese, and obesity induces insulin resistance (6). In general researchers believe that several genetic factors and environments factor can lead to the incidence of diabetic mellitus type II.

The aim of this study is to analyze sequence of TCF7L2 gene in Iraqi diabetic patients using bioinformatics method.

2. Materials and Methods
The study involved 10 Iraqi patients with diabetic mellitus type II, aged form 18 to 70 year-olds, the patients samples were collected from 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 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 manufacturers protocol, and agarose gel electrophoresis used to confirm the presence of the extracted DNA.

The TCF7L2 gene sequences were taken from NCBI database, also the primers was designed for the TCF7L2 gene using two primers and provided by integrated DNA technologies company, Canada. The sequences of both forward and reverse primers are shown in table 1.

Table 1. The sequences of both forward and reverse primers used for TCF7L2 gene amplification

<table>
<thead>
<tr>
<th>Primer</th>
<th>Sequence</th>
<th>Tm(°C)</th>
<th>GC (%)</th>
<th>Product size</th>
</tr>
</thead>
<tbody>
<tr>
<td>Forward</td>
<td>5'-TCATAGGGGTCTGGC TTGGA- 3'</td>
<td>57.8</td>
<td>55</td>
<td>888 bp</td>
</tr>
</tbody>
</table>
The TCF7L2 primers used for amplify TCF7L2 gene by using PCR pre mix kit, Intron/Korea according to the manufactures instruction. The mixture of PCR solution is shown in table 2.

Table 2. The components of PCR solution for amplification of TCF7L2 gene

<table>
<thead>
<tr>
<th>Components</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Taq PCR Pre Mix</td>
<td>5µl</td>
</tr>
<tr>
<td>Forward primer</td>
<td>5 picomols/µl (1µl)</td>
</tr>
<tr>
<td>Reverse primer</td>
<td>5 picomols/µl(1µl)</td>
</tr>
<tr>
<td>DNA</td>
<td>1.5 µl</td>
</tr>
<tr>
<td>Distill water</td>
<td>16.5 µl</td>
</tr>
<tr>
<td>Final volume</td>
<td>25µl</td>
</tr>
</tbody>
</table>

The condition of PCR reaction to amplify TCF7L2 gene shown in table 3.

Table 3. The optimum conditions of detection TCF7L2 gene

<table>
<thead>
<tr>
<th>No.</th>
<th>Phase</th>
<th>Tm(°C)</th>
<th>Time</th>
<th>No. of cycle</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-</td>
<td>Initial Denaturation</td>
<td>95°C</td>
<td>3 min.</td>
<td>1cycle</td>
</tr>
<tr>
<td>2-</td>
<td>Denaturation -2</td>
<td>95°C</td>
<td>45sec</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Annealing</td>
<td></td>
<td>64°C</td>
<td>45sec</td>
</tr>
<tr>
<td>---</td>
<td>-----------</td>
<td>---</td>
<td>------</td>
<td>-------</td>
</tr>
<tr>
<td>3-</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4-</td>
<td>Extension-1</td>
<td></td>
<td>72°C</td>
<td>45sec</td>
</tr>
<tr>
<td>5-</td>
<td>Extension-2</td>
<td></td>
<td>72°C</td>
<td>7min.</td>
</tr>
</tbody>
</table>

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

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

3. Results and Discussion

3.1. Detection of TCF7L2 gene by PCR Technique

The results were showed using two primers that using to amplify the TCF7L2 gene exon as a specific primers, the result of amplification segments of DNA of diabetics mellitus type II patients by primers lead to pure PCR bands with size of 888 bp after electrophoresis on 2% agarose gel at 75 volt for 60 min as shown in figure 1.
Figure 1. The products of PCR gel electrophoresis on 2% agarose, shown clear bands at size 888bp of TCF7L2 gene in Iraqi diabetic’s mellitus type II patients

3.2. Sequence Alignment of TCF7L2 gene

The results of TCF7L2 gene sequences for 10 patients with diabetic mellitus type II were received from Macrogen Company/Korea, and then analyzed using BLAST software, where the results showed mutation in the sequence of gene for all patients as compared to the sequence of gene in NCBI.

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

In second patient sample, mutation appeared in Guanine transversion to Thymine (G to T) at site 103894 and Thymine transversion to Adenine (T to A) at both sites 104074, 104505, 18-Thymine transversion to Adenine (T to A) at sites 104074,104505.

In 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 fourth patient sample, 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 fifth patient sample, 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, 104621.

In sixth patient sample, mutation appeared in Transversion of Thymine to Adenine (T to A) to sites 104074, 104505, 23- Transversion of Thymine to Adenine (T to A) at site 104505.

In seventh patient sample, mutation appeared in Transversion of Adenine to Thymine (A to T) at sites 103996,104201 and 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 eighth patient sample, mutation appeared in Transversion of Thymine to Adenine (T to A) at sites 104074, 104275 and 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, Transition of Cytosine to Thymine (C to T) at site 104283.

In ninth patient sample, mutation appeared in Transversion of Thymine to Adenine (T to A) at sites 104064,104074, 104505 and Adenine to Thymine (A to T) at site 103996.

In tenth patient sample, mutation appeared in Transversion of Thymine to Adenine (T to A) at sites 104064,104074, 104505 and Adenine to Thymine (A to T) at site 103996.

In all patients with T2DM, Transversion mutation has been appeared in the sequence of TCF7L2 gene when the Adenine (A) was inserted instead of Thymine (T) in the site 104074 and 104288 as shown in Figure (2) showing the insertion of Adenine (A) in the sites 104074 and 104288 in the sequence of TCF7L2 gene for a patient with T2DM which compared with the sequence of TCF7L2 gene in the NCBI as a part of results by BLAST software.

Hyperglycemia that can occur via mechanisms such as defeated insulin secretion, insulin resistance, and in enhanced glucose output by the liver is the main indicator by which type 2 diabetes could be characterized. The results of the present study confirmed the importance of mutations of TCF7L2 gene in the prevalence of type 2 diabetes in Iraqi patients.
The mutation in the TCF7L2 gene with Iraqi diabetic mellitus type II patients appeared in all samples in which it indicated that the mutations are one of the causes the T2DM and increasing the risk of diabetic mellitus II and the TCF7L2 gene product is a high mobility group box-containing transcription factor previously implicated in blood glucose homeostasis, these results were in agreement with other studies which showed the mutation in TCF7L2 gene in other people in other countries have a higher risk in T2DM (9, 10, 11). Also the results were in alignment with another study conducted by Grant et al, (10) demonstrating that a mutation in the TCF7L2 gene causing the diabetic mellitus type II and increasing the risk of this disease in which have agreement with other study (12).

In conclusion, the mutation in TCF7L2 gene in Iraqi diabetic mellitus type II patients one of the causing and increasing the risk of T2DM disease.
Figure 2. The results of alignment between the sequence of TCF7L2 gene for T2DM patient (query) and the sequence of TCF7L2 gene in NCBI (subject) by BLAST program, where the insertion mutation appeared between the sites 104074 and 104288 (indicated by the blue arrow).

Reference