The correlation between the gene that encoding Calpain-10 and Type 2 diabetes mellitus in Wasit Province

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Abstract

Type 2 diabetes mellitus (T2DM) is a consequence of several interactions between genetic and environmental agents that lead to impairment of insulin action or insulin resistance. The present study was designed to discover the relationship between calpain-10 gene (exon 8, 14 and 15) and T2DM. A total of 150 subjects, 100 patients with T2DM and 50 healthy control. These topics were genotyped for (3 exon) of calpain-10 utilizing PCR and confirmed by direct sequencing. In sequence analysis of exon eight has been exposed a match in the amino acid sequencing between T2DM and healthy control. But the exon 14 has been detected 2 mutations, the first was (18778 G>A) substitution of the amino acid arginine at site 18778 with lysine, and the frequency rate (20 %). The second mutation was (18787 C>A) substitution of the amino acid proline at site 18787 with histidine, and the frequency rate (40 %). These sequencing exon 15 has been identified one mutation, this mutation was (19690 A>T) substitution of the amino acid serine at site 19690 with threonine, and the frequency rate (46.8 %). In conclusion, our study has been explained that the variation in exon 14 and 15 of calpain-10 might cause increase susceptibility and may be linked with type 2 diabetes mellitus.

Keywords: Calpain-10, Type 2 diabetes mellitus

http://doi.org/10.36295/ASRO.2021.24504

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Volume/Issue: Volume: 24 Issue: 05

Introduction

Diabetes mellitus (DM): It is a heterogeneous and metabolic disorder in which a person is characterized by hyperglycemia, either because the β-cell of pancreas does not produce insulin, or because cells do not respond to the insulin that is produced (1, 2, and 3). DM can be classified into four types: type I diabetes mellitus (T1DM), type II diabetes mellitus (T2DM), gestational diabetes mellitus (GDM) and other types of DM. T2DM is maturity onset Diabetes, and the most common type of diabetes. T2DM is described by either insulin resistance when the body fails to properly use insulin or pancreas does not produce adequate insulin. This type of diabetes, which accounts for about 90-95% of all diagnosed cases of diabetes. T2DM is rise with obesity, age, and lacks of physical activity (4, 5). Genetic factors have an important role in the development of DM. Some forms of this disease resulting from mutations in a single gene and others in multifactorial (6). Based on the role of genetic variables T2DM can be split into two types: monogenic and polygenic (7, 8). Monogenic forms comprises only a small percentage of type 2
diabetes cases (≤10%), are simpler in nature (9). A polygenic form occurs more commonly and accounts for ~90% of all cases. Over than 30 chromosomal regions comprising one or more susceptibility genes have been recognized in diverse populations, have revealed association to different genetic regions (10). Calpain-10 is the first predisposition gene recognized in T2DM. CAPN-10 is a calpain member and is expressed in many human tissues such as pancreas, heart, liver, brain, and kidney (11). Calpain-10 is situate on chromosome 2q37, comprises of 15 exons spanning 31 kb (Figure 1-1). CAPN-10 is consist of domain I, domain II and repeat domain III (12). CAPN-10 is an atypical calpain, domain IV has been substituted by a domain III similar structure. It thus seems that its protease domain is accompanied by a tandem repeat of two domains like to domain III which suggest that CAPN-10 may have different cellular functions including regulated exocytosis in β-cell of pancreas, a key role in regulating glucose homeostasis, play a role in insulin secretion, neuronal functions, intracellular signal transduction, cytoskeletal rearrangements, association with pathophysiological features of T2DM both a rise in sensitivity to insulin and a deficiency in insulin secretion. This also contributes to the breakdown of other proteins and can therefore modulate the activity of other enzymes through its proteolytic function, as well as adjust the apoptosis process (13, 14, 15, 16, 17, 18).

Aims of the study

The current study is designed to identify the variations in calpain-10 gene in type 2 diabetes mellitus.

Materials and Methods

Study designer and samples collection

The present study was included type 2 diabetes patients in AL-Zahra Teaching Hospital, in Wasit Province, Iraq; 100 patients with type 2 diabetes mellitus were taken for this study, 50 males and 50 females with age ranged (40-79) years.

The healthy control in the current study was 50 individuals, 25 males and 25 females with age ranged (40-79) years. All patients and controls were non-suffering hepatitis who were free from any signs and symptoms of liver disease, chronic renal disease, heart disease, smoking, alcohol taking, lipid disorders, diabetes mellitus (for control only), hypertension and other disease.
5 ml of venous blood samples were drawn from type 2 diabetes mellitus. Also, 5 ml of venous blood samples were drawn from healthy people to be used as control. All obtained samples were packaged in numbered EDTA anticoagulant for subsequently Genomic DNA Extraction.

**DNA Extraction**

The DNA extracted from blood specimen by Presto™ Minig DNA provided by Genaid, Canada, Kit. Then agarose gel electrophoresis was appropriated to check the presence and complete of DNA extraction (19).

**PCR protocols**

PCR was performed by AccuPower PCR Premixkit (Bioneer, Korea) with specific primers were utilized in this study and their sizes were displayed in Table (1-2). After the components have been added the reaction was performed according to (Horikawa et al, 2003) with some modification (table 1-3). Then the PCR products of (calpain -10) gene were sending to Source Bioscience Company for sequencing. Sequences were opened utilizing chromase pro software and aligned using BLAST (basic local alignment search tool provided by NCBI or using standalone Bio Edit package, ver 7.2.5). First ATG represent the start of exon.

**Table (1-2):- The primer sequences**

<table>
<thead>
<tr>
<th>Name of primers</th>
<th>Sequence 5´-3´</th>
<th>Size(bp)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Calpain-10</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>exons 8</td>
<td>forward 5´-GGATATGCCCCGCTGCTCCTCA-3´ reverse 5´-GGTGGACCTCCCGTCTGATGC-3´</td>
<td>531</td>
<td>14</td>
</tr>
<tr>
<td>exons14</td>
<td>forward 5´-GGTTCTTAGTTGGCAGCTTCTT-3´ reverse 5´-TTCAGGAGGGAATTGCAATGAC-3´</td>
<td>363</td>
<td>14</td>
</tr>
<tr>
<td>exons15</td>
<td>forward 5´-TCCTCAGGCCAGCTGCCCATTTG-3´ reverse 5´-TTCGGGCTCTGCCAAACTGGGT-3´</td>
<td>401</td>
<td>14</td>
</tr>
</tbody>
</table>

**Table (1-3):- PCR Programs**

<table>
<thead>
<tr>
<th>Step</th>
<th>Temperature</th>
<th>Time</th>
<th>Cycle</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-denaturation</td>
<td>95 ºC</td>
<td>5 minutes</td>
<td>1 cycle</td>
</tr>
<tr>
<td>Denaturation</td>
<td>95 ºC</td>
<td>20 second</td>
<td></td>
</tr>
<tr>
<td>Annealing</td>
<td>58 ºC for exon 8 and 14.67 ºC for exon 15</td>
<td>20 Second</td>
<td>25-35 cycle</td>
</tr>
<tr>
<td>Extension</td>
<td>72 ºC</td>
<td>30 Sec-1min/kb</td>
<td></td>
</tr>
<tr>
<td>Final Extension</td>
<td>72 ºC</td>
<td>Optional</td>
<td>1 cycle</td>
</tr>
</tbody>
</table>
**Statistical application**

DNA sequence analysis software

The genotype and allelic frequency between T2DM and control subject being calculated by Haplotag software. P<0.5 was take into account to be the last limit of significance.

**Results**

**Genotype of calpain -10 (exon 8)**

Data present in calpain -10 (exon 8) has been revealed in the patients T2DM and healthy control (figure 1-2). In sequence analysis, our study has been exposed a match in the amino acid sequence between T2DM and healthy control.

**Genotypes of calpain -10 (exon14)**

Sequence analysis of calpain -10 (exon14) has been discovered in the patients T2DM and healthy control summarized in (Table 1-5). We were found two different mutation in the patients T2DM, the first was (18778 G>A) substitution of the amino acid arginine at site 18778 with lysine, and the frequency rate 20 %. The second mutation was (18787 C>A) substitution of the amino acid proline at site 18787 with histidine, and the frequency rate 40 % (Figure1-3).

**Table (1-5). Genotypes distribution of calpain -10 (exon14)**

<table>
<thead>
<tr>
<th>Exon 14</th>
<th>Mutation</th>
<th>A.A change</th>
<th>Frequency of T2DM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>18778 G&gt;A</td>
<td>Arg&gt;Lys</td>
<td>20 %</td>
</tr>
<tr>
<td></td>
<td>18787 C&gt;A</td>
<td>pro&gt;His</td>
<td>40 %</td>
</tr>
</tbody>
</table>

**Genotypes of calpain -10 (exon15)**

The result of calpain -10 (exon15), has been revealed in the patients T2DM and healthy control (Table 1-6). In sequence analysis, we were displayed one mutation in the patients T2DM, this mutation was (19690 A>T) substitution of the amino acid serine at site 19690 with threonine, and the frequency rate 46.8 % (figure 1-4).

**Table (1–6) Genotype distribution of calpain -10(exon15)**

<table>
<thead>
<tr>
<th>Exon 15</th>
<th>Mutation</th>
<th>A.A change</th>
<th>Frequency of T2DM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>19690 A&gt;T</td>
<td>Ser&gt;Thr</td>
<td>46.8 %</td>
</tr>
</tbody>
</table>

**Discussion**

Our result has been explained that the sequencings of calpain-10 encoding region (exons 14 and 15) were performed to identify these variants. Results showed three novel mutations distributed throughout the entire coding region. The first mutation in exon 14 was result in substitution of the amino acid arginine at site 18778 to lysine, with the frequency rate 20 %. The second mutation in exon 14 was substitution of the amino acid proline at site 18787 with histidine, and the frequency rate 40 %.
to histidine, with the higher frequency rate (40%). The third mutation in exon 15 was substitution of the amino acid serine at site 19690 to threonine, with the higher frequency rate (46.8%). But in sequence analysis of exons 8 was observed a match in the amino acid sequence between T2DM and healthy control.

The current study has been displayed that the amino acid arginine in exon-14 has chemical properties similar with lysine according to (hydrophilic, basic, side chain form ionic bonds and in physiological pH have a charge of +1) but differ from chemical structure. The amino acid proline has chemical properties diverge with histidine from where (polar, side chain, hydrophilic and chemical structure).

As we know that the amino acid serine in exon -15 has chemical properties like with threonine according to (polar, uncharged, and hydrophilic) but differ from chemical structure and side chains. The side chains in these groups possess a chain of functional groups however, most have at least one atom (nitrogen, oxygen or sulfur) with electron pairs available for hydrogen bonding to water and other molecules.

Our result has been exposed that widely diversity of amino acid changes may be lead to altering the protein structure and thus function. These fluctuations cause mutation and effect on insulin action and secretion because the role of CAPN-10 is involved in insulin secretion of pancreatic β-cells (20, 21, 22 and 23) as well as insulin action and hepatic glucose production.

Azad, (2018)24 has been shown that genes are made up of promoter regions and alternating regions of introns (noncoding sequences) and exons (coding sequences). The production of a functional protein involves the transcription of the gene from DNA into RNA, the removal of introns and splicing together of exons, the translation of the spliced RNA sequences into a chain of amino acids, and the posttranslational modification of the protein molecule.

The greatest essential posttranslational alteration of amino acids in humans is reversible adding of a phosphate molecule to the portion hydroxyl of the R groups of serine and threonine. This technique is recognized as phosphorylation and is utilize to regulate the activity of proteins in their functioning in the cell.

So the changeable in the R groups of amino acids may be effect on phosphorylation and thus mutation.

Song et al, (2004)25 has been explained that the chromosomes in all human are matching through the sorts, but the alleles and their rates at many positions vary vastly among people groups. This be able due to migration or mutation but usually occurs gradually, in small augmentations. Gene-gene or gene-environmental exchanges could due to the varying genetic effects of calpain-10 detected in diverse populations.

Recently, many studies have shown that the variation in the gene encoding Calpain-10 is associated with T2DM (26, 27 and 28). Therefore this is leading to the variation in the disease susceptibility.

On the other hand, Matthew et al, (2013)29 has been revealed if the calpain-10 gene’s function deteriorates due to the gene’s mutation, it is more likely to cause T2DM.

Johnson et al, (2004)30 has been suggested that CAPN-10 acts to induce apoptosis in response to fatty acids. It may enhance lipotoxic activation of apoptosis in T2D islets effecting overall insulin secreting function.
Salih et al. (2021): Correlation between gene encoding Calpain-10 and T2 DM
© Annals of Tropical Medicine & Public Health
DOI: http://doi.org/10.36295/ASRO.2021.24504

Figure (1-2):- Sequencing of calpain -10 (exon-8)

Figure (1-3):- Sequencing of calpain -10 (exon-14)

Figure (1-4):- Sequencing of calpain -10 (exon-15)
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