A study on the association of TCF7L2 rs11196205 (C/G) and CAPN10 rs3792267 (G/A) polymorphisms with type 2 diabetes mellitus in the South Western of Iran

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Abstract
Background: Type 2 diabetes mellitus is a multifactorial and heterogenic disease with a complex etiology. In recent decades the association of a large number of genes has been shown with T2DM. CAPN10 gene was the first T2DM candidate gene identified through genome-wide screening and positional cloning, and among all identified genes until now, TCF7L2 gene has shown most association with T2DM. The aim of this study was to investigate the association between TCF7L2 rs11196205(C/G) and CAPN10 rs3792267 (G/A) with T2DM in a subset of Iranian population from Khuzestan province. It should be noted that this is the first report of TCF7L2 polymorphism rs11196205 with T2DM in Iran.

Subjects and methods: A case-control association study was performed using 150 T2DM patients and 150 controls. Genotyping for TCF7L2 rs11196205 was done by Tetra-Primer ARMS-PCR and for CAPN10 rs3792267 was done by PCR-RFLP Technique.

Results: Statistical analyses were carried out using SPSS version 16. In examining TCF7L2 rs11196205 based on the genotype GG, results for CG genotype were, 95%CI = (0.5–1.7), OR = 0.92, P-value = 0.79 and for genotype CC were, 95%CI = (0.94–3.92), OR = 1.92, P-value = 0.07. in examining CAPN10 rs3792267 based on the genotype AA, results for GG genotype were, 95%CI = (0.55–6.8), OR = 1.93, P-value = 0.31 and for genotype GA were, 95%CI = (0.43–5.64), OR = 1.55, P-value = 0.5. So, in both polymorphisms, none of the alleles or genotypes had significant statistical differences between case and control groups (P > 0.05).

Conclusion: Our results showed that TCF7L2 rs11196205 and CAPN10 rs3792267 (SNP-43) polymorphisms are not associated with the risk of T2DM in the studied population.

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1. Introduction

One of the most common form of diabetes, constituting ~95% of the diabetic population is Type 2 diabetes mellitus. It is a heterogeneous group of disorders which is associated by hyperglycemia that can occur through mechanisms such as impaired insulin secretion, insulin resistance in peripheral tissues and increased glucose output by the liver [1,2]. Genetic and environmental factors have a strong role in the manifestation of T2DM as a complex genetic disorder. It is becoming an epidemic with increasing prevalence throughout the world [3]. As reported, the prevalence of diabetes has been estimated about 400 million people in the world while >150 million of them seem to be still undiagnosed. It is predicted that this prevalence by 2035 reaches to around 600 million [4]. In Iran, the prevalence of T2DM was about 24% as showed by a systematic review between years 1996 and 2004, and the risk was 1.7% greater for women than for men. According to this report the prevalence of T2DM in Iran seems to be highest amongst developing countries. Previous reports on total urban population of Middle East countries show the prevalence of T2DM as 3.4% in Sudan, 20% in United Arab Emirates, 8.5% in Bahrain, and 12.1% in India [5]. Changing patterns of diet, as well as decrease in physical activity practice
can be one of the best likely explanations for the growing increase in T2DM prevalence observed over the past decades. However, it is suggested but only in the presence of genetic risk factors, these lifestyle changes may lead to T2DM [6]. Great effort has been made to identify the genes which are associated with T2DM, and so far, several novel susceptibility genes have been uncovered across several ethnic groups, Using GWAS and candidate gene approaches [7,8].

Common variants in TCF7L2 (transcription factor 7-like 2) gene have been identified as the strongest genetic risk factors for T2DM in multiple ethnic groups [9]. Initially, strong association of TCF7L2 with T2DM was reported in Icelandic people and then subsequent replication has been found in U.S and Danish populations [3]. TCF7L2 gene which spans about 215.9 Kb with 17 exons is located on chromosome 10q25.3. This gene encodes a transcription factor which is involved in the Wnt signaling pathway. TCF7L2 gene which affects insulin secretion and glucose production, is expressed in many tissues including fat, liver and pancreatic islets of Langerhans. It has been shown that TCF7L2 gene plays a central role in coordinating the expression of proinsulin and its subsequent processing to form mature insulin [10,11]. rs11196205 at the TCF7L2 gene is located in intron 4 [12].

Among the >40 genes related to T2DM (OMIM, 2013), the association of CAPN10 (calpain-10) gene was initially reported with diabetes in Mexican-Americans people [13]. Association of CAPN10 gene polymorphisms in development of T2DM in multiple ethnic groups in several case-control studies has been indicated. CAPN10 gene is located on chromosome 2q37.3, and with 15 exons spanning 31 kb, which encodes a 672 amino-acid intracellular protease [14]. Similar to other calpains, CAPN10 consists of an isoform-specific large subunit and a common small subunit, and was shown to act as intracellular calcium-dependent cysteine proteases in calcium-regulated signaling pathways [15]. Although CAPN10 is highly expressed in heart, brain, liver and kidneys and pancreas, its main role is in tissues such as skeletal muscle. Howbeit insulin secretion, insulin action, insulin stimulated glucose transport and insulin stimulated glycogen synthesis are the significant metabolic activities related to the disease of an individual, evidences from the studies show that there are susceptibility genes controlling these metabolic activities and CAPN10 and its variants contribute to the metabolic activities and T2DM [16]. rs3792267(G/A) at the CAPN10 gene is located in the third intron [14].

The aim of this study was investigation of association between rs11196205(C/G) at TCF7L2 gene and rs3792267(G/A) at CAPN10 with T2DM in a population from Khuzestan province, Iran.

2. Subjects and methods

2.1. Subjects

The type of study was case-control and a total of 300 individuals, including 150 unrelated adult T2DM patients (77 men and 73 women; age 52.51 ± 9.15 years) who were selected from among those referred to the Diabetes Clinic and Golestan Hospital in Ahvaz, and 150 unrelated controls (71 men and 79 women; age 56.49 ± 7.18 years) who were selected from among people referred to Valiasr Hospital in Khoramshahr and Golestan Hospital of Ahvaz (Table 1). T2DM criteria were based on the standard of ADA. According to which FPG ≥ 126 mg/dl or 2-hPG (2h Plasma Glucose) ≥ 200 mg/dl or RBG (Random Blood Sugar) ≥ 200 mg/dl to be characterized as diabetic. People with diabetes were chosen as samples that were taking medicines to treat diabetes. For control individuals, lack of history of diabetes in the subjects and among their first-degree relatives was carefully monitored. The case and control subjects were living in Khuzestan province. The project has been approved by special committee genetic division of Shahid Chamran university for considering the ethical issues of patients and controls who participated in sampling. Before blood sampling, both diabetic patients and healthy subjects signed the consent form to participate in this study. The present study has been carried out in accordance with the Code of Ethics of the world Medical Association (Declaration of Helsinki) for experiments in humans.

2.2. DNA extraction and genotyping

Leukocyte Genomic DNA was extracted from whole blood samples by non-enzymatic salting out method. All participants were genotyped for two SNPs including TCF7L2 rs11196205 (C/G) and CAPN10 rs3792267 (G/A).

2.2.1. TCF7L2 rs11196205

Genotyping for rs11196205 was carried out using the TETRA ARMS PCR technique and following primers were used: forward inner primer: 5’-CTGAAACGTTCACACATTITATATAACTGCC-3’ and reverse inner primer: 5’-CAACATTAACTCTCTCTACACACGC-3’ and forward outer primer: 5’-TAGATTTGCTCCTTTTTTGTCTCTGAC-3’ and reverse outer primer: 5’-TAAACATGCTACCTTTAGCC-3’.

Master Mix PCR was provided from Ampliqon Company. Using Bio-Rad thermal cycler, each PCR was performed in a volume of 25 μl, including 2 μl of genomic DNA, 0.75 μl of each external primer, 2 μl of each internal primer, 5 μl of distilled water, and 12.5 μl of Master mix. The size of DNA fragments amplified with these four primers for TCF7L2 (434 bp control fragment, 253 bp C allele, 235 bp G allele), was suitable for separation on 3% agarose gel. To determine the size of DNA fragment in gel the 50 bp Ladder was used.

2.2.2. CAPN10 rs3792267

rs3792267 was genotyped using PCR-RFLP Technique. For this purpose, the part of CAPN10 gene that contains intended polymorphism, was amplified by PCR technique using the primer pair 5’-CACGCTTGCTGAAAGAATGC-3’ (forward) and 5’-TGATTTGCTCCTTTTTTGTCTCTGAC-3’.

Table 1

Demographic and clinical characteristics of the cases and controls.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Cases</th>
<th>Controls</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>77(51.3%)</td>
<td>71(47.3%)</td>
<td>P &gt; 0.05</td>
</tr>
<tr>
<td>Female</td>
<td>73(48.7%)</td>
<td>79(52.7%)</td>
<td>P &gt; 0.05</td>
</tr>
<tr>
<td>Ethnicity</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arab</td>
<td>98(65.3%)</td>
<td>96(64%)</td>
<td>P &gt; 0.05</td>
</tr>
<tr>
<td>non-Arab</td>
<td>52(34.7%)</td>
<td>54(36%)</td>
<td>P &gt; 0.05</td>
</tr>
<tr>
<td>Mean Age (years)</td>
<td>52.51 ± 9.15</td>
<td>56.49 ± 7.18</td>
<td>P &lt; 0.01</td>
</tr>
<tr>
<td>Diabetes duration (years)</td>
<td>6.32 ± 3.97</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Mean B.M.I (kg/m²)</td>
<td>28.9 ± 5.02</td>
<td>26.49 ± 4.52</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
<td>FPG (mg/dl)</td>
<td>172.15 ± 81.4</td>
<td>91.37 ± 6.29</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
<td>HBA1c(%)</td>
<td>8.31 ± 2.33</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>TG (mg/dl)</td>
<td>144.76 ± 55.63</td>
<td>122.81 ± 56.96</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
<td>TC (mg/dl)</td>
<td>177.9 ± 48.54</td>
<td>184.21 ± 53.18</td>
<td>P &lt; 0.001</td>
</tr>
</tbody>
</table>
CATGGTCTGTAGCAC-3' (reverse). PCR amplification was performed using the following reagents: 1.5 µl of genomic DNA, 0.75 µl of each primer, 9.5 µl of distilled water, and 12.5 µl of Master Mix. PCR amplification of CAPN10 gene produced a 141 bp DNA fragment. In order to confirm the PCR amplification, gel electrophoresis (1/5% agarose gel and DNA safe stain) was done. For RFLP analysis 8 µl of PCR product was digested by 4U of a restriction enzyme Mph1103I (Fermentas) at 37 °C for 16 h in 20 µl reaction mixture that contained 10x Buffer R.

The G-allele was not cleaved by Mph1103I and gave a 141 bp band and the A-allele was cleaved into two bands 118 and 23 bp. It should be noted that the 23 bp fragment cannot be seen due to its small size in the gel. The digested products were separated by running on 2/5% agarose gel with DNA size marker of 50 bp and observed using a UV transilluminator.

2.3. Statistical analysis

All statistical analyses were performed using SPSS v.16.0 software. Data for continuous variables were expressed as mean ± standard deviation (SD). The differences in continuous data between T2DM patients and control participants were assessed using Mann-Whitney test. Categorical data such as allele and genotype frequencies are shown as number (percentage) and analyzed by χ² test. For evaluating of association of TCF7L2 rs11196205 and CAPN10 rs3792267 polymorphisms with T2DM, Odds Ratios (ORs), 95% confidence intervals (95% CIs) and corresponding P values were calculated using logistic regression. P < 0.05 was considered statistically significant.

Variables with normal distribution are presented as mean ± SD or percentage, p values <0.05 are considered as significant. BMI: body mass index, FPG: fasting plasma glucose, HbA1c: hemoglobin A1c, TG: triglyceride, TC: total cholesterol.

3. Results

The clinical and biochemical characteristics of the participants are summarized in Table 1. Our results showed statistically significant differences for diabetes criteria between control and T2DM patients (P < 0.001). The average age of control group was significantly higher than the average age of patients (P < 0.01), and the mean of BMI and triglycerides were significantly higher in patient group than the control group (P < 0.001), but there were no statistically significant differences between control and T2DM patients for sex, ethnicity (Arab and non-Arab) and total cholesterol (P > 0.05). The results of PCR product of TETRA ARMS-PCR for TCF7L2 rs11196205 and PCR amplification of CAPN10 gene and digestion results of PCR products are shown in Figs. 1–3.

To determine the association of TCF7L2 rs11196205 and CAPN10 rs3792267 polymorphisms with T2DM, we analyzed the distribution of allelic and genotypic frequencies between patients and healthy controls for both SNPs and our results revealed that there was no statistically significant differences for allele and genotype frequencies of TCF7L2 rs11196205 or CAPN10 rs3792267 polymorphisms between subjects with T2DM and non-diabetic subjects. The allele and genotype frequencies of TCF7L2 rs11196205(C/G) are shown in Tables 2 and 3. The prevalence of genotype

![Fig. 1. Agarose gel electrophoresis (3%) of PCR product of Tetra-Primer ARMS-PCR for TCF7L2 rs11196205.](image1)

![Fig. 2. Electrophoresis of PCR Product 141 bp for the amplification of the CAPN10 rs3792267 on 1.5% agarose gel.](image2)

![Fig. 3. 2.5% agarose gel showing results for CAPN10 rs3792267 in some individuals; GG homozygote is observed as a fragment of 141 bp and AA homozygote is observed as a fragment of 118 bp and heterozygote (GA) is observed as two fragments (141 and 118 bp).](image3)

Table 2

<table>
<thead>
<tr>
<th>Allele</th>
<th>Controls</th>
<th>Cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>150(50%)</td>
<td>173(57.66%)</td>
</tr>
<tr>
<td>G</td>
<td>150(50%)</td>
<td>127(42.33%)</td>
</tr>
</tbody>
</table>
Genotype frequency of TCF7L2 rs11196205 in the cases and controls.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Controls</th>
<th>Cases</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total (N%)</td>
<td>Female (N%)</td>
</tr>
<tr>
<td>CC</td>
<td>28 (18.66%)</td>
<td>12 (8%)</td>
</tr>
<tr>
<td>CG</td>
<td>94 (62.66%)</td>
<td>56 (37.33%)</td>
</tr>
<tr>
<td>GG</td>
<td>28 (18.66%)</td>
<td>11 (7.33%)</td>
</tr>
</tbody>
</table>

Allele frequencies of CAPN10 rs3792267 polymorphism in the cases and controls.

<table>
<thead>
<tr>
<th>Allele</th>
<th>Controls</th>
<th>Cases</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total (N%)</td>
<td>Female (N %)</td>
</tr>
<tr>
<td>G</td>
<td>233 (77.66%)</td>
<td>245 (81.66%)</td>
</tr>
<tr>
<td>A</td>
<td>67 (22.33%)</td>
<td>55 (18.33%)</td>
</tr>
</tbody>
</table>

Table 4

Genotype frequency of TCF7L2 rs11196205 in the cases and controls.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Controls</th>
<th>Cases</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total (N%)</td>
<td>Female (N %)</td>
</tr>
<tr>
<td>CC</td>
<td>28 (18.66%)</td>
<td>12 (8%)</td>
</tr>
<tr>
<td>CG</td>
<td>94 (62.66%)</td>
<td>56 (37.33%)</td>
</tr>
<tr>
<td>GG</td>
<td>28 (18.66%)</td>
<td>11 (7.33%)</td>
</tr>
</tbody>
</table>

Genotype frequency of CAPN10 rs3792267 in the cases and controls.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Controls</th>
<th>Cases</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total (N%)</td>
<td>Female (N %)</td>
</tr>
<tr>
<td>CC</td>
<td>90 (60%)</td>
<td>55 (36.66%)</td>
</tr>
<tr>
<td>GA</td>
<td>53 (35.33%)</td>
<td>20 (13.33%)</td>
</tr>
<tr>
<td>AA</td>
<td>7 (4.66%)</td>
<td>4 (2.66%)</td>
</tr>
</tbody>
</table>

TCF7L2 gene clearly showed the largest effect size with an odds ratio of 1.37 and according to meta-analyses that were conducted by Florez and also Cauchi et al., the TCF7L2 gene is a strong susceptibility gene for T2DM in different ethnic groups [17,21,22]. In our study on TCF7L2 rs11196205(C/G) polymorphism, the frequency of CC genotype in patient group was higher than healthy group, while frequency of CG and GG genotype in healthy subjects was higher than the patient group. Also, frequency of C allele was higher in the patient group. But in general, contrary to the results of many studies conducted in other populations, TCF7L2 rs11196205 did not show significant association with T2DM in population of Khuzeistan province. This is the first report of association study of TCF7L2 rs11196205 polymorphism with T2DM in Iran. Doing a cross-national research with larger sample sizes would help us to achieve a more accurate result.

CAPN10 was the first T2DM candidate gene identified through genome-wide screening and positional cloning [23,24]. Specific CAPN10 variants, such as rs3792267 reportedly confers increased risk of T2DM in some populations [25–31]. But the association of CAPN10 rs3792267 with T2DM in some other studies has not confirmed [14,32–38]. So far, two studies have been conducted on the association of rs39792267 with T2DM in Iran; a study in 2012 by Bahrani and colleagues was conducted on the population of West Azerbaijan, and G allele as a risk factor for T2DM in this population was identified. In another study by Maleki and his colleagues on the Kurdish ethnicity in Ilam city in 2014, the association between rs39792267 and T2DM was not confirmed [37,38]. This is the first study performed on the association of CAPN10 rs3792267 with T2DM in the population of Khuzeistan province from southwest Iran. In this study, CAPN10 rs3792267 did not show either allelic or genotypic association with T2DM.

5. Conclusion

The study of the role of genetic factors in development of heterogeneous multifactorial diseases, such as T2DM, is very complicated, because the genetic analysis of this disease, in addition to identifying and estimating the contribution of different variants in different genes that are effective, requires to understand the interactions between these genes and variants as well as their cumulative effects. Our results suggested that TCF7L2 rs11196205 and CAPN10 rs3792267 polymorphisms are not associated with the risk of T2DM in Khuzeistan province, Iran. The difference and diversity in results of mentioned studies and the present study may be influenced by many factors such as ethnic differences (the role and effect of genetic varieties in different ethnic groups), environmental factors and epigenetic effects, sample size, diagnostic method,
differences in sampling methods (in terms of demographic characteristics such as age and sex) and other factors.

**Conflict of interest**

The authors declare no conflict of interest.

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**References**


