Association of rs12255372 (TCF7L2) and D76N (PDX-1) Polymorphisms with Type 2 Diabetes in a Population Living in Northeast Iran

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Abstract

The aim of this study was to investigate whether the rs12255372 (TCF7L2) and D76N (PDX-1) polymorphisms are associated with type 2 diabetes mellitus (T2DM) in Mashhad, northeast Iran.

A hundred twenty seven patients with T2DM and 71 non-diabetic controls in Mashhad were genotyped by PCR-RFLP and ARMS-PCR methods. Single nucleotide polymorphisms (SNPs) were confirmed by sequencing in some samples and allelic and genotypic frequencies were then analyzed in each group.

The T-allele of the SNP rs12255372 of TCF7L2 (OR = 2.70, 95% CI = 1.12–6.49, P = 0.027) and the A-allele of PDX-1 D76N (OR = 3.93, 95% CI = 1.60–7.68, P = 0.002) were significantly associated with an increased risk of T2DM. The rs12255372 SNP of TCF7L2 and D76N of PDX-1 genes may confer susceptibility to T2DM in the population living in Mashhad.

Keywords: D76N, rs12255372, PDX-1, TCF7L2, Type 2 diabetes mellitus

Introduction

The dramatic increase in the prevalence of T2DM is a serious threat to public health. The prevalence of T2DM in Iran is 4%–4.5% and the total cost of T2DM per patient in 2012 was estimated to be over 5 million Rials. Genome-wide association studies have identified many insulin secretion-related genetic variants associated with T2DM, such as transcription factor 7-like 2 (TCF7L2; OMIM 602228) and pancreatic duodenal homeobox (PDX-1; OMIM 600733) genes. TCF7L2 is an important transcription factor in the canonical WNT signaling pathway, which is involved in pancreas development, islet β-cell function, and insulin production and secretion. There is cross talk among the WNT, FOXO, and insulin signaling cascades in pancreatic β-cells. PDX-1 is a homeodomain transcription factor required for pancreas development and the transcriptional regulation of specific genes such as insulin in pancreatic β-cells.

The TCF7L2 gene (10q25) has emerged as the strongest T2DM susceptibility gene in multiple populations. A meta-analysis published in 2013 detected a significant association between the TCF7L2 polymorphism rs12255372 and T2DM. To date, multiple mutations have been identified in the PDX-1 gene (13q12.1) in patients with diabetes. The most frequently reported polymorphism in patients with diabetes is D76N (GAC→AAG), which causes an amino acid change of aspartic acid to asparagine.

In this study, the associations between TCF7L2 rs12255372 and PDX-1 D76N variants with T2DM were investigated in an Iranian population who has been living in Mashhad for at least 15 years prior to the study. To our knowledge, these SNPs have not been previously tested in the northeastern Iranian population.

Materials and Methods

The study consists of 127 patients (54 males / 73 females) referring to Danesh Amooz Hygiene Center and 71 healthy controls (38 males / 33 females) randomly selected based on the WHO Diabetes Criteria, with fasting plasma glucose concentrations ≥7.0 mmol/L. Before their participation, the purpose and risks of the study were carefully explained and informed consents were obtained. The protocol was approved by the ethics committee at Ferdowsi University of Mashhad. Fasting glucose and triglyceride serum levels were determined by enzymatic colorimetric assays using standard kits (Pars Azmoon, Mashhad, Iran).

DNA analysis

Genomic DNA was isolated from leukocytes using Fermentas DNA extraction kit based on a standard salting out protocol. rs12255372 (TCF7L2) and D76N (PDX-1) were genotyped by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) and amplification refractory mutation system-PCR (ARMs-PCR), respectively (Table 1). The normal and mutant alleles of rs12255372 were respectively digested to three (162, 97, and 14 bps) and four (145, 97, 17, and 14 bps) fragments following Tsp 509I (Fermentas, Germany) digestion. Selected normal and mutant samples were also verified by sequencing at Macrogen sequencing facility (Korea).

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Accepted for publication: 20 May 2015
Statistical analysis
Data were analyzed with the SPSS program (version 18.0). To compare quantitative data in groups of carriers with different genotypes, the unpaired Student’s *t*-test was used. The Hardy-Weinberg equilibrium is considered as a quality control measure for the data. The odds ratios (OR) and 95% confidence intervals (CI) were used to estimate the strength of association between different groups and alleles or genotypes of rs12255372 and D76N variants. Characteristics and polymorphic variants were assessed using two-way ANOVA. The observed correlations were then adjusted for patients’ conventional risk factors by analysis of covariance (ANCOVA) using BMI, serum glucose, or triglyceride levels.

Results
Table 2 shows the clinical and biochemical characteristics, allelic and genotypic frequencies, and estimates of relative risks for the TCF7L2 rs12255372 and PDX-1 D76N SNPs in T2DM and control subjects. A comparison between diabetic and normal subjects showed that serum glucose and triglyceride were significantly higher in diabetic subjects (*P < 0.05*). PDX-1 D76N genotype in controls was in Hardy-Weinberg equilibrium. Both allelic variants showed strong association with T2DM (*P < 0.05*). There was no association between TCF7L2 rs12255372 and PDX-1 D76N variants with BMI, serum glucose, or triglyceride levels.

Discussion
A meta-analysis by Peng, et al. showed that rs12255372 was associated with diabetes risk in the global population, with OR = 1.33, and 95% CI = 1.27–1.40. In our study, the allele T of rs12255372 was associated with a higher diabetes risk (OR = 2.73, 95% CI = 1.14–6.57) when compared with that reported by Alami, et al. (OR = 1.458, 95% CI = 1.108–1.918) and Shokuhi, et al. (OR = 2.02, 95% CI = 1.3–3.13) in their diabetic populations from the north (Golestan Province) and west (Ilam and Kermanshah Provinces) regions of Iran, respectively. The PDX1 D76N variant has been extensively studied, but the reproducibility of its association with T2DM has varied. In the present study, the allele A of PDX-1 D76N showed an association with diabetes risk.

Table 1. Primer sequences, PCR reactions and conditions for the amplification of TCF7L2 (rs12255372) and D76N (PDX-1) variants.

<table>
<thead>
<tr>
<th>Polymorphisms</th>
<th>Primers (5’-3’)</th>
<th>Amplicon length (bp)</th>
<th>PCR Reaction Components</th>
<th>PCR Conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs12255372</td>
<td>F: CAACTGAGATTCCAGAATTGCCCT&lt;br&gt;R: CTGTCTATTGGCATCTAAATGG</td>
<td>273</td>
<td>0.2 mM dNTPs, 3 mM MgCl₂, 20 pmol of each primer, 1000 ng genomic DNA, 2.5 u Taq DNA polymerase</td>
<td>95°C/2 min, 30 cycles of (95°C/20 s, 60°C/1 min, 72°C/30 s) and elongation at 72°C for 5 min.</td>
</tr>
<tr>
<td>D76N</td>
<td>F: CGCTGGCTGTGCTTCCCTCTGA&lt;br&gt;R: TGAAGGTGCGCCACCGCGGGGTC (N)²&lt;br&gt;R: AAGGTGGCCACCGGGGGCT (M)²</td>
<td>406</td>
<td>0.2 mM dNTPs, 3 mM MgCl₂, 8 pmol of each primer, 200 ng genomic DNA, 1.5 u Taq DNA polymerase, 7% DMSO</td>
<td>94°C/12 min, 35 cycles of (95°C/40 s, 70°C/1 min, 72°C/1 min) and elongation at 72°C for 5 min.</td>
</tr>
</tbody>
</table>

* M = mutant-specific primer; N = normal-specific primer.

Table 2. Clinical and biochemical characteristics, allelic and genotypic frequencies, and estimates of relative risks for the TCF7L2 rs12255372 and PDX-1 D76N SNPs in T2DM and control subjects.

<table>
<thead>
<tr>
<th>Subjects</th>
<th>TCF7L2 (Case /Control)</th>
<th>PDX-1 D76N (Case /Control)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Frequency, N (%) (Case /Control)</td>
<td>OR (95% CI), p</td>
</tr>
<tr>
<td>Age (year)</td>
<td>BMI (kg/m²)</td>
<td>FBS (mmol/l)</td>
</tr>
<tr>
<td>T/T</td>
<td>11 (8.7) / 2 (2.8)</td>
<td>3.67 (0.96–14.10), 0.058</td>
</tr>
<tr>
<td>G/T</td>
<td>26 (20.4) / 7 (9.9)</td>
<td>2.45 (1.08–5.57), 0.032</td>
</tr>
<tr>
<td>G/G</td>
<td>90 (70.9) / 62 (87.3)</td>
<td>Reference</td>
</tr>
<tr>
<td>T</td>
<td>48 (19) / 11 (8)</td>
<td>2.70 (1.12–6.49), 0.027</td>
</tr>
<tr>
<td>G</td>
<td>206 (81) / 131 (92)</td>
<td>Reference</td>
</tr>
</tbody>
</table>
on a French population, which showed that D76N mutation was more prevalent and associated with an overall relative risk of 12.6 for developing diabetes and with decreased glucose-stimulated insulin-secretion in non-diabetic subjects.7

The important finding of this study is that the T-allele of TCF7L2 rs12255372 and the A-allele of PDX-1 D76N were significantly associated with an increased risk of T2DM in this Iranian population living in Mashhad. However, further studies on larger populations from different parts of the city are still required.

Conflict of interest
There is no conflict of interest.

Funding
The authors express their gratitude to Ferdowsi University of Mashhad (Grant No. 17758/3) and Institute of Biotechnology (Grant No. 17873) for supporting this study.

References