Brief Report

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

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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.[1,2] 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.[3,4] 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.[5] 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.[6]

The *TCF7L2* gene (10q25) has emerged as the strongest T2DM susceptibility gene in multiple populations.[3,5] A meta-analysis published in 2013 detected a significant association between the *TCF7L2* polymorphism rs12255372 and TD2M.[3] 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→AAC), which causes an amino acid change of aspartic acid to asparagine.[4,7]

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.

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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).

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

Polymorphisms	Primers (5'-3')	Amplicon length (bp)	PCR Reaction Components	PCR Conditions
rs12255372	F: CAACTGGATTTCAGAATTGTCCCT R: CTGTCTATTTGGCATTCAAATGG	273	0.2 mM dNTPs, 3 mM $MgCl_2$, 20 pmol of each primer, 1000 ng genomic DNA, 2.5 u *Taq* DNA polymerase	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.
D76N	F: CGCTGGCTGTGGGTTCCCTCTGA R: TGAAGGTGCGCCACCGCGGGGTC (N)[a] R: AAGGTGCGCCACCGCGGGGCT (M)[a]	406	0.2 mM dNTPs, 3 mM $MgCl_2$, 8 pmol of each primer, 200 ng genomic DNA, 1.5 u *Taq* DNA polymerase, 7% DMSO	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.

[a] M = mutant-specific primer; N = normal-specific primer.

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 genotyping. Odd 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. The significance of association between clinical characteristics and polymorphic variants was assessed using two-way ANOVA. The observed correlations were then adjusted for patients' conventional risk factors by analysis of covariance (ANCOVA) using BMI, glucose and triglyceride as covariates.

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 significantly associated with susceptibility to T2DM in the global population, with OR = 1.33, and 95% CI = 1.27–1.40.[3] 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.[8,9] 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 increased risk of T2DM. This finding was similar to a report

Table 2. Clinical and biochemical characteristics, allelic and genotypic frequencies, and estimates of relative risks for the *TCF7L2* rs12255372 and *PDX-1* D76/N SNPs in T2DM and control subjects.

Subjects	(Case /Control)				TCF7L2 rs12255372			PDX-1 D76/N		
	Age (year)	BMI (kg/m^2)	FBS (mmol/l)	TG (mmol/l)	Allele/ Genotype	Frequency, N (%) (Case / Control)	OR (95% CI), p	Allele/ Genotype	Frequency, N (%) (Case / Control)	OR (95% CI), p
127/71	59.4/51.7	28/27.9	10.94/5.48	1.96/1.49	T/T	11 (8.7) / 2 (2.8)	3.67 (0.96–14.10), 0.058	A/A	- / -	-
					G/T	26 (20.4) / 7 (9.9)	2.45 (1.08–5.57), 0.032	G/A	71 (55.9) / 14 (19.7)	5.09 (2.71–9.55), $<10^{-4}$
					G/G	90 (70.9) / 62 (87.3)	Reference	G/G	56 (44.1) / 57 (80.3)	Reference
					T	48 (19) / 11 (8)	2.70 (1.12–6.49), 0.027	A	71 (28) / 14 (10)	3.50 (1.60–7.68), 0.0018
					G	206 (81) / 131 (92)	Reference	G	183 (72) / 128 (90)	Reference

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.

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References

1. Lotfi MH, Saadati H, Afzali M. Prevalence of diabetes in people aged ≥30 years: the results of screening program of Yazd Province, Iran, in 2012. *J Res Health Sci*. 2013; **14**: 88 – 92.
2. Ezzatabadi MR, Rahmanian M, Jamali E, Tafti AD. Estimating per capita direct costs associated with type 2 diabetes: a cross-sectional study in Yazd, Iran. *Iran J Diabetes Obes*. 2013; **5:** 60 – 66.
3. Peng S, Zhu Y, Lü B, Xu F, Li X, Lai M. TCF7L2 gene polymorphisms and type 2 diabetes risk: a comprehensive and updated meta-analysis involving 121 174 subjects. *Mutagenesis*. 2013; **28:** 25 – 37.
4. Macfarlane WM, Frayling TM, Ellard S, Evans JC, Allen LI, Bulman MP, et al. Missense mutations in the insulin promoter factor-1 gene predispose to type 2 diabetes. *J Clin Invest*. 1999; **104:** R33 – R39.
5. Ip W, Chiang YT, Jin T. The involvement of the wnt signaling pathway and TCF7L2 in diabetes mellitus: the current understanding, dispute, and perspective. *Cell Biosci*. 2012; **2:** 28.
6. Khoo C, Yang J, Weinrott SA, Kaestner KH, Naji A, Schug J, et al. Research resource: the pdx1 cistrome of pancreatic islets. *Mol Endocrinol*. 2012; **26:** 521 – 533.
7. Hani EH, Stoffers DA, Chèvre JC, Durand E, Stanojevic V, Dina C, et al. Defective mutations in the insulin promoter factor-1 (*IPF-1*) gene in late-onset type 2 diabetes mellitus. *J Clin Invest*. 1999; **104:** 41 – 48.
8. Alami FM, Ahmadi M, Bazrafshan H, Tabarraei A, Khosravi A, Tabatabaiefar MA, et al. Association of the TCF7L2 rs12255372 (G/T) variant with type 2 diabetes mellitus in an Iranian population. *Genet Mol Biol*. 2012; **35:** 413 – 417.
9. Shokouhi S, Delpisheh A, Haghani K, Mahdizadeh M, Bakhtiyari S. Association of rs7903146, rs12255372, and rs290487 polymorphisms in TCF7L2 gene with type 2 diabetes in an Iranian Kurdish ethnic group. *Clin Lab*. 2013; **60:** 1269 – 1276.