

Association of Transcription Factor 7-Like 2 (*Tcf7l2*) Gene Haplotypes with the Risk of Type 2 Diabetes Mellitus in Iran

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Abstract: Genetic polymorphisms of the transcription factor 7-like 2 (*TCF7L2*) are strongly associated with the risk of type 2 diabetes mellitus (T2DM) in several populations. In this study, we aimed to explore the effect of the *TCF7L2* SNP rs7903146 (C/T) polymorphism in Golestan province, northeast of Iran. In this case-control association study, we studied 236 unrelated patients with T2DM and 255 healthy controls from Golestan province, northeast of Iran. The rs7903146 (C/T) polymorphism was genotyped using the PCR-RFLP method. Using the PHASE software haplotypes of this variant and rs12255372 were inferred. The T allele of *TCF7L2* rs7903146 (C/T) was associated with T2DM in the study population ($p = 0.007$; OR= 1.681, 95%CI: 1.150-2.459). CC and CT genotypes were found to be significantly different between the two groups ($p=0.045$; OR= 1.499, 95% CI: 1.008-2.229). The highest risk was observed under the co-dominant model ($p = 0.0001$; OR= 2.991, 95% CI: 1.671-5.352). Haplotype analyses also showed a higher distribution of haplotype TT in the T2DM patients than the control subjects [32.2 % vs. 21.1 %, ($P < 0.000$; OR= 1.77, 95% CI: 1.31-2.40)]. Our data prove that rs7903146 (C/T) variant of the *TCF7L2* gene is associated with T2DM in Iranian population.

Key words: *TCF7L2* gene • Rs7903146 (C/T) variant • Type 2 diabetes mellitus (T2DM)

INTRODUCTION

Type 2 diabetes mellitus (T2DM) is a highly inheritable metabolic disorder of polygenetic nature characterizing by impaired insulin secretion, insulin resistance in peripheral tissues and increased glucose output by the liver [1, 2]. Several studies have shown strong association between T2DM and variation in the transcription factor 7-like 2 (*TCF7L2*) gene [3]. *TCF7L2* is an important transcription factor in the Wnt/beta-Catenin signaling pathway which is involved in glucose homeostasis through the regulation of pro-glucagon

gene expression [4, 5]. Grant and colleagues have reported the association of a common microsatellite (DG10S478) within intron 3 of *TCF7L2* with T2DM [2]. There are at least four well-studied single nucleotide polymorphism (SNP) markers in the human *TCF7L2* gene, which are associated with T2DM, viz., rs7903146, rs7901695, rs12255372 and rs11196205 [6].

In our previous study, an rs12255372 variant of the *TCF7L2* gene was found to be associated with T2DM [7]. Since several genome-wide association studies (GWAs) independently confirmed the strong associations of SNP rs7903146 (C/T) in the *TCF7L2* locus with T2DM [8-11]

and also that phylogenetic studies have demonstrated that the genetic variants within the *TCF7L2* gene, especially rs7903146 T, are major genetic risk factors for T2DM [6,12], we launched the present study to investigate the association of rs7903146 (C/T) variant and the haplotypes resulted from the alleles of this variant with those of rs12255372, with T2DM. In a couple of recent reports from other regions of Iran the association between a *TCF7L2* rs7903146 (C/T) variant and T2DM has been shown [13,14].

The aim of present study was to examine the possible impact of *TCF7L2* rs7903146 (C/T) variant on the pathogenesis of T2DM in Golestan province, northeast of Iran.

MATERIAL AND METHOD

Study Subjects: The study population consisted of 236 unrelated T2DM patients and 255 control subjects. All diabetic patients (n = 236) were recruited in collaboration with Golestan University of Medical Sciences and Gorgan clinic of diabetes (Golestan province, northeast of Iran). Control subjects (n = 255) were recruited from the same area and were age-matched with the case population. Diagnosis of T2DM patients was based upon the American Diabetes Association criteria (15) with fasting plasma glucose ≥ 126 mg/dl, 2-h plasma glucose ≥ 200 mg/dl during an oral glucose tolerance test.

All enrolled patients gave written informed consent for participation in the study and Patients with age of onset <40 years were excluded. Anthropometric measurements including weight, height and waist were obtained using standardized techniques. Individual weight and heights were obtained from all the subjects and the body mass index (BMI) were calculated as the weight in kilograms divided by the square of height in meters. HbA1c levels were determined by using turbidometric inhibition immunoassay (Tina Quant, Roche, Basel, Switzerland).

BMI, waist circumference and HbA1c were measured in T2DM patients and controls. The study was approved by the Ethics committee of Golestan University of Medical Sciences (GOUMS) (No: 1090).

Blood Sample Collection and DNA Extraction: Peripheral blood was drawn from T2DM patients and controls in EDTA sterile tubes and stored at -20 C before DNA extraction. Genomic DNA was extracted from whole blood using the phenol/chloroform procedure (16).

PCR-RFLP and Genotyping: The PCR based RFLP method was employed for genotyping of the rs7903146 (C/T) polymorphism. For detection of the *TaaI* (*HpyCH4III*) restriction fragment length polymorphism, a 136-base pair fragment was amplified with forward primer (5'- AAG AGA AGA TTC CTT TTT AAA TGG TG -3') and backward primer (5'- CCT CAT ACG GCA ATT AAA TTA TAC A -3').

PCR conditions were as follows: 5 min at 94°C, followed by 35 cycles of 30 s at 94°, 30 s at 56°C and 30 s at 72°C. A final extension was performed at 72°C for 10 min. PCR products after treatment with *TaaI* (*HpyCH4III*) restriction enzyme were separated on 3.5% agarose gel and visualized after ethidium bromide staining. Some of genotyping results were validated by direct sequencing.

Statistical Analyses: Genotype frequencies were tested for Hardy-Weinberg equilibrium by χ^2 analysis. Logistic regression model was used to adjust the age and BMI. Mean \pm SD and relative frequency were used for quantitative and qualitative traits, respectively. SHAPIRO-WILK test was used to compare normality of quantitative traits in both diabetic and control subjects. The association of rs7903146 (C/T) SNP with T2DM in the matched case-control subjects was tested using multinomial regression model analysis followed by calculation of Odds ratios (ORs) with 95% confidence intervals (CIs). Mean of quantitative traits in both groups was compared by t-test. All statistical analyses were performed using SPSS Statistical version 18.0 for Windows (SPSS, Chicago, IL, USA). A *P* value of <0.05 was considered to be significant. The associations of SNPs and possible two-variant haplotypes of *TCF7L2* with T2DM were analyzed using the PHASE software (17).

RESULTS

A case-control association study was conducted, including 236 T2DM patients and 255 control subjects, both aged above 40 years. Both groups were of Fars origin and from the same region. The mean age was 51.79 \pm 9.48 and 49.27 \pm 8.40 for T2DM patients and control subjects, respectively.

Thirty five subjects (22 control subjects and 13 T2DM patients) were excluded, through unavailability of individual genomic DNA, thus leaving 233 T2DM patients and 233 control subjects for genotyping. Genotype frequencies did not differ from the expected Hardy-Weinberg equilibrium ratios.

Table 1: The *TCF7L2* rs12255372 polymorphism allele and genotype frequencies in T2DM patients and Control subjects

Genotype	Control subjects(N=233)	T2DM * patients (N=233)	OR (95% CI)	p value†
CC (%)	112 (48.0)	80 (34.3)	Reference	-
CT (%)	99 (42.4)	106 (45.4)	1.499(1.008-2.229)	0.045
TT (%)	22 (9.4)	47 (20.1)	2.991(1.671-5.352)	0.0001
TT+CT (%)	121 (51.9)	153 (65.6)	1.77 (1.21-2.57)	0.003
CC+CT (%)	211 (90.5)	186 (79.8)	2.42 (1.40-4.17)	0.001
C allele (%)	323 (69.3)	226 (57.1)	Reference	-
T allele (%)	143 (30.7)	200 (42.9)	1.681(1.150-2.459)	0.007

* Type 2 diabetes mellitus. † P value of <0.05 was considered as significant.

Table 2: Association analysis of *TCF7L2* haplotypes with T2DM

Haplotype	T2DM * patients (%)	Control subjects (%)	OR [95% CI]	p value†
C-G	220 (50.0)	271 (59.6)	-	-
C-T	29 (6.5)	42 (9.2)	-	-
T-G	49 (11.1)	45 (9.9)	-	-
T-T	142 (32.2)	96 (21.1)	1.77 (1.31-2.40)*	<0.000

OR, Odds ratio, * Type 2 diabetes mellitus, † P value of <0.05 was considered as significant

Note: The calculated OR value shows the ratio of T-T to non-T-T haplotypes in the case groups vs. the control group. In other words, the total of C-G, C-T and T-G serve as the reference.

The T allele of *TCF7L2* rs7903146 (C/T) was associated with T2DM in the study population ($p = 0.007$; OR= 1.681, 95%CI: 1.150-2.459) (Table 1). Significant difference was also observed for the CC and CT genotype frequencies between T2DM patients and control subjects ($p=0.045$; OR= 1.499, 95%CI: 1.008-2.229).

Dominant and recessive models were considered to investigate which model would fit the effect of *TCF7L2* rs7903146 (C/T). Assuming the recessive model, comparison between T2DM patients and control subjects showed a significant association for rs7903146 (C/T) variant TT vs. CT + CC ($p= 0.001$; OR= 2.42, 95%CI: 1.4-4.17) and T vs. C allele ($p= 0.007$; OR= 1.681, 95%CI: 1.150-2.459) (Table 1).

In the dominant model, CC genotype frequency of both T2DM patients and control subjects was compared to the CT+TT genotypes. This indicated a significant difference between T2DM patients and control subjects ($p= 0.003$; OR= 1.77, 95%CI: 1.21-2.57). Also, the TT and CT genotypes showed a significant difference in their frequencies ($p = 0.0001$; OR= 2.991, 95% CI: 1.671-5.352). Thus, the highest risk was observed under the co-dominant model ($p= 0.0001$; OR= 2.991, 95% CI: 1.671-5.352) (Table 1).

The association between haplotypes of the two SNPs of *TCF7L2* including rs7903146 (C/T), rs12255372 (7) and T2DM were explored in our study population (Table 2). Haplotype TT had a higher distribution among T2DM patients than the controls [32.2 % vs. 21.1 %, (P

<0.000; OR=1.77, 95% CI: 1.31-2.40)]. No significant association was observed between other haplotypes and T2DM (Table 2).

DISCUSSION

The polymorphism of *TCF7L2* SNP rs7903146 is the strongest variant associated with T2DM [2,8,11] and has been confirmed in numerous populations throughout the world [8, 10, 11, 13, 14, 18-35]. In this replication study, we aimed to investigate the association between the *TCF7L2* rs7903146 (C/T) variant and T2DM in Golestan province, northeast of Iran. Our case-control study, along with the previous reports from different region of Iran, prove that the rs7903146 (C/T) variant of the *TCF7L2* gene is associated with T2DM in Iranian population. A significant association between the T allele of *TCF7L2* rs7903146 (C/T) and T2DM was observed in our study population ($p= 0.007$; OR= 1.681, 95%CI: 1.150-2.459) (Table 1). This finding is consistent with the previous reports from Iran and other populations, except for Arab population of Persian Gulf [36, 37]. The minor T allele frequency for rs7903146 was 30.7% in the control subjects and 42.9% in T2DM subjects of this study (Table 1). The frequency of the minor T allele in the control subjects was consistent with that of rs7903146 T in the populations of Icelandic, Palestinian, Danish, Asian-Indian, Dutch, UK-resident South Asians and Emirati populations (30.4, 29.3, 29, 26.1, 28, 29 and 37.2%, respectively)

[12, 30, 35, 37-39], but strikingly different from that reported in the Japanese (4.2%) and Chinese (~2%) populations [6, 32]. Moreover, the frequency of the minor T allele (30.7%) in our control subjects was slightly higher than that previously reported by Amoli *et al* in southeast of Iran (29%) [13] and lower than that recently reported by Palizban *et al* in the centre of Iran (34.4%) [14]. The variability in the allele frequency, which probably reflects different ethnic backgrounds, would partly account for the discrepancies found in the results among different studies. Other factors include small sample size, age of subjects [37] and effects of environmental factors, such as life-style. In our study, the frequency of TT genotype was higher in T2DM patients compared to the control subjects (20.1% vs. 9.4%) and with a nearly two times higher OR=2.991(95%CI 1.671-5.352), thereby implying a possible gene dosage effect. While the T allele of the rs7903146 variant shows an OR= 1.681(95%CI 1.150-2.459), data from our previous study on rs12255372 [7] obtained an OR= 1.458 for allele T ($p=0.007$, OR 1.458, 95%CI 1.108-1.918). Haplotype analysis of these two variants of the *TCF7L2* gene also shows that haplotype TT increases the risk of developing T2DM in the Iranian population (32.2% vs. 21.1%, $P<0.000$; OR= 1.77 95%CI: 1.31-2.40), in contrast with previous reports on Arab populations of Persian Gulf [36, 37]. Therefore, it could be concluded that the T alleles of the two variants in combination only slightly increase the risk and that rs7903146 could separately serves in risk assessment for T2DM.

CONCLUSION

In Summary, from the obtained data suggest that the *TCF7L2* gene is a major determinant of T2DM in the Iranian population. Further studies will be necessary to understand any possible effect of the studied variants on insulin action. Also, the possibility that other variants of *TCF7L2* may affect the risk of the disease in our population cannot be excluded.

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