



Research paper

Single and multi-locus association study of TCF7L2 gene variants with susceptibility to type 2 diabetes mellitus in an Iranian population

Saeed Kalantari^a, Alireza Sharafshah^b, Parvaneh Keshavarz^{b,*}, Arash Davoudi^c, Razie Habibipour^b

^a Department of Endocrinology, Guilan University of Medical Sciences, Rasht, Iran

^b Cellular and Molecular Research Center, Faculty of Medicine, Guilan University of Medical Sciences, Rasht, Iran

^c Division of Cytogenetic, Medical Genetic Laboratory of Dr. Keshavarz, Rasht, Iran



ARTICLE INFO

Keywords:

Type 2 diabetes mellitus
TCF7L2
Variant
Haplotype

ABSTRACT

Prior studies indicated that some of transcription factor 7-like 2 (TCF7L2) gene variants such as rs7903146, rs12255372 and rs11255372 are constantly associated with Type 2 diabetes mellitus (T2DM) in various populations and ethnic groups. The purpose of this study was to assess the association between TCF7L2 variants (rs7903146, rs11196205, and rs11255372) and T2DM by TaqMan assay. Statistical analysis was performed through SNPalyze and SPSS. Significant associations of rs7903146 ($P = 1.9 \times 10^{-7}$), and rs11255372 ($P = 2.98 \times 10^{-10}$) both under a dominant model were found. Based on allele frequency, there was a significant difference between the two study groups at rs7903146 and rs12255372 variants ($P = 6.8 \times 10^{-10}$, and $P = 9.3 \times 10^{-11}$, respectively). Two haplotypes including Hap-1 (C-G-G) and Hap-2 (T-G-T) indicated a significant difference between the two study groups ($P = 1.174 \times 10^{-9}$ and $P = 7.432 \times 10^{-9}$ respectively). In conclusion, rs7903146 and rs12255372 were significantly associated with T2DM in the specified Northern Iranian population.

1. Introduction

Diabetes is a major health problem all around the world (Yang et al., 2015). About 220 million people worldwide have diabetes (Safarpour et al., 2015). It is estimated that the individuals affected by type 2 Diabetes mellitus (T2DM) accounting for 90% of diagnoses, will increase to 300 million by the year 2025 (Yang et al., 2015). Therefore, diabetes could be introduced as a major problem expanding rapidly through global health (Vijan, 2010). Many people can blame their T2DM, partly on their genes. For over two decades there was a hope that identifying the “guilty” genes would help improve the understanding of the fundamental pathophysiology of this common and important disorder (Karnes et al., 2013). The risk of T2DM is highly influenced by inheritance. Genetic susceptibility to the common form of T2DM, appears polygenic which means that, a number of variants are involved with a modest effect on the risk of disease in an individual person. Despite important progresses in understanding the genetic

determinants of relatively rare monogenic forms of diabetes, the pace of definitive identification of genes that increase the risk of common T2DM has evidently decreased (Ouhaibi-Djellouli et al., 2014).

Recently, transcription factor 7-like 2 (TCF7L2), located on cytogenetic site 10q25.3, has been identified as a major susceptible gene of T2DM. Single nucleotide polymorphisms (SNPs) of TCF7L2 have been consistently associated with T2DM in various ethnic groups (Muendlein et al., 2011). Specific associated variants increase the risk of T2DM 1.5-fold in heterozygotes and 2.4-fold in homozygotes, corresponding to a population attributable risk of 21%. This makes TCF7L2 variants as the strongest known genetic risk factors for T2DM (Yan, 2009). Among TCF7L2 gene variants, rs7903146 and rs12255372 were found to be most significantly associated with T2DM risk (Florez et al., 2006; Saxena et al., 2006; Tsai et al., 2014). Furthermore, rs290487 and rs11196205 as the TCF7L2 variants have been linked with impaired glucose metabolism and increased diabetes risk (Florez et al., 2006; Körner et al., 2007; Muendlein et al., 2011). After a strong linkage

Abbreviations: T2DM, Type 2 diabetes mellitus; GWAS, genome-wide association studies; SNP, single nucleotide polymorphism; TCF7L2, transcription factor 7 like 2; HbA1c, hemoglobin A1c; FBS, fasting blood sugar; mmol/l, milliMol per liter; mg/dl, milligrams per deciliter; PCR, polymerase chain reaction; LD, linkage disequilibrium; χ^2 , chi-square; OR, odds ratio; AOR, adjusted odds ratio; ANOVA, one-way analysis of variance; df, degree of freedom; GDM, Gestational Diabetes Mellitus; NEO, Netherlands Epidemiology of Obesity

* Corresponding author at: Cellular and Molecular Research Center, Faculty of Medicine, Guilan University of Medical Sciences, Rasht 13377-41937, Iran.

E-mail address: keshavarz@gums.ac.ir (P. Keshavarz).

<https://doi.org/10.1016/j.gene.2019.01.040>

Received 24 October 2018; Received in revised form 4 January 2019; Accepted 22 January 2019

Available online 15 February 2019

0378-1119/ © 2019 Published by Elsevier B.V.

signal was mapped to chromosome 10q in a Mexican-American population, *TCF7L2* was discovered as a T2DM susceptible gene. This region was later fine-mapped in an Icelandic population and confirmed in The United States and Danish cohorts, where the risk locus was found to be located in intron 3 of the *TCF7L2* gene. There are remarkable indications that suggest this gene may play a critical role in cancer as well as in diabetes (Yan, 2009). Therefore, in T2DM individuals, diagnosis of high-risk groups and prognosis of disease progression is an emerging challenge. The current study aimed to investigate the association of the *TCF7L2* SNPs including rs7903146, rs12255372 and rs290487 with T2DM in northern Iranians.

2. Methods

2.1. Subjects

A total of 537 unrelated Iranian patients with T2DM (386 female, 151 male) from different areas of Guilan province were randomly selected during 2014 to 2018 from the outpatient clinics at Razi Hospital Diabetes Center of Guilan University of Medical Science. 441 healthy controls (261 females, 180 males) were selected from people with normal fasting glucose referred to the medical lab for clinical testing. All subjects were unrelated and older than 35 years. All volunteers who met the inclusion criteria for participating in this study were Iranian and resided in Guilan province. Inclusion criteria for subjects with T2DM was as follows: having glycosylated hemoglobin value (HbA1c) ranging from 53 to 108 mmol/mol (7.0–12.0%) and a fasting blood sugar (FBS) level ranging from 7.8 to 14.0 mmol/l (140–252 mg/dl). All patients were diagnosed based on the World Health Organization criteria or using medication for diabetes treatment (Alberti and Zimmet, 1998). The subjects underwent routine medical check-ups, and individuals with no clinical evidence of major diseases were enrolled as the control group. The inclusion criteria for subjects without T2DM were as follows: subjects older than 50 years who had FBS levels lower than 5.6 mmol/l (100 mg/dl), normal HbA1c level < 42 mmol/mol (6%) and no family history of T2DM. The exclusion criteria for both study groups included not being in the aforementioned ranges of HbA1c, FBS, familial history of T2DM, and also samples with bad DNAs (low concentrations and pale qualifications). This case-control study was approved by the ethical committee for human genome/gene research at the Guilan University of Medical Science, and a written informed consent was obtained from each participant. High molecular weight genomic DNA was isolated from peripheral blood according to standard salting-out method (Keshavarz et al., 2014) and analyzed by electrophoresis in 1% agarose gels stained with ethidium bromide. DNA concentration was determined using a Nanodrop (ND-1000, ABI).

2.2. Genotyping of *TCF7L2* variants

Three variants were selected for genotyping purposes: rs7903146, rs12255372 and rs290487. Genotyping were performed using the TaqMan allelic discrimination assay (Applied Biosystems, Inc., Foster city, CA). The TaqMan genotyping reaction was amplified on a real time PCR system, ABI 7300, (95 °C for 10 min, 92 °C for 15 s and 60 °C for 1 min, for 45 cycles). Fluorescence was detected on the same ABI 7300.

2.3. Linkage disequilibrium (LD) analysis

LD analysis were performed among 3 variants rs7903146, rs12255372 and rs290487 of *TCF7L2*. Pairwise delta (correlation coefficient between SNPs) was estimated from genotypes and the results were visualized by the SNPalyze program (ver. 8.0, Dynacom, Japan).

2.4. Haplotype analysis

To estimate the potential effects of three SNPs combinations on the

risk of T2DM, haplotype analysis was performed on rs7903146, rs12255372 and rs290487. Significant haplotypes selected with frequencies higher than 5%. Haplotype analysis among case and control groups were performed by the maximum-likelihood method with an expectation-maximization algorithm. Permutation *p*-values were calculated by comparing haplotype frequencies between cases and controls on the basis of 10,000 replications.

2.5. Statistical analysis

All analyses and statistical computations were performed using SPSS program (ver.19, <http://www.spss.com/>) and SNPalyze software (ver.8.0, Dynacom, Japan). Genotype and allele frequencies of *TCF7L2* variants in the case and control groups were compared, and tested using a Pearson χ^2 -statistic. Deviations from Hardy-Weinberg equilibrium (HWE) were tested using χ^2 goodness-of-fit test. Analyses were also performed assuming dominant (major allele homozygotes vs. heterozygotes + minor allele homozygotes, co-dominant (heterozygotes vs. major allele homozygotes + minor allele homozygotes), and recessive (minor allele homozygotes vs. major allele homozygotes + heterozygotes) models of inheritance and crude odd ratios (ORs), their 95% CI ranges and corresponding *P*-values were calculated using both SNPalyze and the Web-Assotest program (available at <http://www.ekstroem.com/>) (Table 2). Logistic regression was done to estimate both unadjusted and age-, gender- and body mass index (BMI) - adjusted ORs. Analysis of variance (ANOVA) or the unpaired two-tailed Student's test was performed to quantitatively compare clinical data among populations or genotypes. The significance level for statistical tests was chosen to be lower than 0.05. To detect an association, statistical power was determined with the PS power sample-size program (<http://biostat.mc.vanderbilt.edu/twiki/bin/view/Main/PowerSampleSize>). The present study (a total sample size of 537 cases and 441 controls) had a statistical power of > 90% to detect an association with an OR of 1.5 at *P* = 0.05, for alleles with > 10% frequency.

3. Results

3.1. Clinical characteristics of study groups

The control group were consisted of 175 men and 266 women. Mean (Mean ± Standard Deviation) clinical characteristics of healthy people in the variables were reported as: Body mass index (BMI) 26.56 ± 4.1, Age: 56.2 ± 12.37, the level of fasting blood glucose (FBG): 92.4 ± 6.3, Diagnostic blood pressure (DBP): 7.8 ± 0.9 and systolic blood pressure (SBP): 12.5 ± 1.7 respectively. In this group, 16.7% of individuals were obese, 44.7% were overweight, and 38.6% were normal. Among the 537 T2DM patients, 135 were male and 402 were female. Their means were as follows: BMI 28.5 ± 5, age: 52.4 ± 9.1, the level of fasting blood glucose: 150.3 ± 63.30, DBP: 7.6 ± 0.9, SBP: 12.5 ± 1.5 and HbA1c 7.1 ± 5.7 respectively. In this group 34.6% were obese, 41.2% were overweight, 24.2% were normal. 68.1% of T2DM individuals participating in the project were first-degree relatives with diabetes. Blood sugar in 31.7% of T2DM individuals was controlled with insulin injections. The mean age of onset in diabetes patients were 44.7 ± 12.2. Based on the distributions of subjects, gender, age, fasting plasma glucose, high blood pressure, and SBP were statistically significant between the two study groups (*P* < 0.001). On the other hand, no significant difference was found in mean of DBP (*P* = 0.645) and BMI (*P* = 0.943) (Table 1).

Variants which were at a Hardy-Weinberg equilibrium and had Minor Allele Frequency (MAF) > 5%, were analyzed by *D'* and *r*² values. Among these three variants of the *TCF7L2*, all three SNPs were in Hardy-Weinberg equilibrium and their MAFs were > 5% and had been analyzed to measure |*D*| and *r*². According to the analysis results, *D'* indicated that none of the studied SNPs were in a significant LD among both T2DM individuals and control subjects (data are not shown).

Table 1
Clinical characteristics of the study subjects.

Characteristics	Control	Case	P-value
Female	266	402	†
Male	175	135	
Age (years)	56.8 ± 12.4	52.4 ± 9.1	†
Fasting blood glucose (ml/dl)	92.4 ± 6.3	150.3 ± 63.30	†
Diastolic blood pressure (mm Hg)	7.8 ± 0.9	7.6 ± 0.9	0.645
Systolic blood pressure (mm Hg)	12.5 ± 1.7	12.5 ± 1.5	0.020
BMI (kg/m) ²	26.56 ± 4.1	28.5 ± 5.0	0.943
HbA1c (%)	-	7.1 ± 5.7	-
Age at onset of diabetes (years)	-	44.7 ± 12.2	-
Age > 50 years	-	262(65.83%)	-
Age ≥ 50 years	-	104(26.13%)	-
Unknown age	-	32(8.04%)	-
Family history	-	-	-
First-degree relatives (%)	-	267(68.1%)	-
Insulin therapy (%)	-	116(31.7%)	-
Obesity (%)	66(16.6%)	126(34.3%)	†
High blood pressure	87(24.6%)	68(19%)	†

Data are expressed as means ± SD, P values refer to independent t-test and Fisher's exact test, F.B.S.: Fasting blood sugar, SBS: Systolic blood pressure, DBP; Diastolic blood pressure. † refers to p-values lower than 0.001.

3.2. Comparison of genotype and haplotype frequencies between patients with T2DM and healthy subjects

Statistical analysis on the associations of rs7903146 and rs11196205 with T2DM between the two study groups represented powerful significant differences under a dominant model of inheritance for both of them ($P = 1.9 \times 10^{-7}$, OR = 0.50; 95%CI [0.38–0.65]). The genotype frequencies of rs11255372 in the two study groups were also strongly significant under a dominant model of inheritance ($P = 2.98 \times 10^{-10}$, OR = 0.43; 95%CI [0.33–0.55]).

Among haplotypes including three variants (rs7903146, rs11255372, rs11196205) there were eight haplotypes with frequencies higher than 1%. Haplotype analysis was performed with SNPalyze software. Among these haplotypes, only two haplotypes with frequencies higher than 5% including Hap-1 (C-G-G) and Hap-2 (T-G-T) indicated significant differences between two study groups ($P = 1.174 \times 10^{-9}$ and $P = 7.432 \times 10^{-9}$, respectively) (Table 3). Permutation test of this haplotypes is also statistically valuable (Table 3). The clinical characteristics of the subjects with and without T2DM are summarized according to three genotypes of TCF7L2 variants using one-way ANOVA test in Table 4. Three genotypes of rs7903146, among control group showed a much smaller difference in frequency of sex ($P = 0.055$). In addition, significant differences were observed in control group with respect to age ($P = 0.027$), systolic ($P = 0.001$) and diastolic blood pressure ($P = 0.02$) within three genotypes. Significant differences were observed among genotypes of rs11255372 in terms of HbA1c ($P = 0.03$). Furthermore, among the subjects without T2DM statistically significant differences were also witnessed in terms of HbA1c ($P = 0.03$) (Table 4).

4. Discussion

The aim of the current study was to investigate the association of the TCF7L2 gene variants with susceptibility to T2DM in a Northern Iranian population. 537 T2DM patients and 441 control subjects were statistically evaluated in a case-control association. In this study, three variants (rs7903146, rs11196205 and rs12255372) of TCF7L2 and their haplotypes were analyzed. Two SNPs, rs7903146 and rs12255372 indicated a strong significant associations with T2DM in the specified Northern Iranian population.

Previous studies have investigated the role of TCF7L2 on the risk of T2DM and related traits including insulin resistance and obesity.

Table 2
The association analysis of TCF7L2 gene variant among T2DM patients and controls subjects.

	Genotype (%)			Allele (%)		P-value		Dominant model		Co-dominant model		Recessives model	
	MM	Mm	mm	Major	Minor	genotype	Allele	OR (95% CI)	P-value	OR (95% CI)	P-value	OR (95% CI)	P-value
rs7903146	Case 155(29.2)	241(45.5)	134(25.3)	0.52	0.48	3.1×10^{-8}	6.8×10^{-10}	0.50 (0.38–0.65)	1.9×10^{-7}	1.65 (1.38–1.97)	0.0001	0.47 (0.32–0.66)	7.1×10^{-6}
Control	187(44.5)	173(41.2)	60(14.3)	0.51	0.49	0.9511	0.7563	0.50 (0.38–0.65)	1.9×10^{-7}	1.01 (0.83–1.23)	0.915	1.06 (0.65–1.66)	0.7826
rs11196205	Case 238(48.4)	207(42.1)	47(9.5)	0.69	0.31	4.3×10^{-10}	9.3×10^{-11}	0.43 (0.33–0.55)	2.98×10^{-10}	1.85 (1.53–2.23)	0.0001	0.50 (0.348–0.70)	1.03×10^{-4}
Control	230(52.6)	171(39.1)	36(8.2)	0.72	0.28								
rs11255372	Case 150(28.3)	271(51.2)	108(20.4)	0.54	0.46								
Control	212(48.1)	180(40.8)	49(11.1)	0.68	0.32								

Number of subjects with each genotype and number of alleles (frequency in %). OR; Odds ratio, CI; confidence interval. ORs for different modes of inheritance were calculated using the Web-Assotest program and SNPalyze (ver.8.5). MM, Mm, and mm are Major Homozygote (major allele homozygotes vs. heterozygotes + minor allele homozygotes), Heterozygote (heterozygotes vs. major allele homozygotes + minor allele homozygotes), and minor homozygote (minor allele homozygotes vs. major allele homozygotes + heterozygotes), respectively. The significance level of P is < 0.05 .

Table 3
Haplotype frequencies and its correlation with the incidence of T2DM.

TCF7L2	SNPs			Frequency		χ^2	p-value	Permutation-P-value
	rs7903146	rs11196205	rs12255372	Case	Control			
Hap-1	C	G	G	0.3019	0.4346	37.0127	1.174×10^{-9}	0
Hap-2	T	G	T	0.2796	0.1692	33.4183	7.432×10^{-9}	0
Hap-3	C	C	G	0.1232	0.1477	2.5064	0.1134	0.205
Hap-4	T	C	T	0.085	0.07	1.5142	0.2185	0.344
Hap-5	T	G	G	0.0808	0.0603	3.07	0.0797	0.142
Hap-6	C	G	T	0.0575	0.0497	0.5781	0.447	0.562
Hap-7	T	C	G	0.035	0.042	0.6479	0.4209	0.511
Hap-8	C	C	T	0.037	0.0265	1.7136	0.1905	0.342

Three SNPs (including rs7903146, rs11196205, and rs12255372) used to analyze haplotypes. Eight Haplotypes with a frequency of > 1% was found.

According to the studied variants in the past decades, *TCF7L2* has had a perceptible effect in the associated study researches. Zhang et al., studied the association of a common variant of the *TCF7L2* (rs12255372 [T/G]) with type 2 diabetes risk, among Caucasians. They reported that the frequencies of the T-allele were significantly higher among cases rather than control subjects; each copy of the T-allele was associated with a 1.32-fold ($P = 0.0002$) and 1.53-fold ($P < 0.0001$) increasing the risk of T2DM among women and men, respectively (Zhang et al., 2006). Lyssenko et al., found that rs12255372 and rs7903146 of *TCF7L2* are strongly associated with T2DM risk. Risk alleles of this gene were associated with dysfunction in pancreatic β -cells in all subjects (Lyssenko et al., 2007). Villareal et al., reported that the *TCF7L2* variant rs7903146 had an effect on the risk of T2DM among the American population. The results supported that the response of β -cell to oral glucose was 50% lower (47 ± 4 vs. $95 \pm 15 \times 109 \text{ min}^{-1}$; $P = 0.01$) among subjects with risk-conferring *TCF7L2* genotypes compared with control subjects (Villareal et al., 2010). In 2011, an analysis including single locus study of *TCF7L2* was linked to T2DM coronary atherosclerosis. They showed that *TCF7L2* variants rs7903146, rs12255372, and rs11196205 are significantly associated with a geographical diagnosis of coronary artery disease (CAD), specifically in patients with T2DM. *TCF7L2* therefore appeared as a genetic link between diabetes and atherosclerosis (Muendlein et al., 2011). Assmann et al., studied the association of rs7903146, and other SNPs of *TCF7L2* with T2DM in Southern-Brazil. The frequency of the minor allele was 38% in T2DM group and 31% in non-T2DM subjects which was significantly associated with T2DM risk under a dominant model of inheritance (Assmann et al., 2014). rs12255372 and rs7903146 are reported to have strong associations with T2DM among subjects from Iceland, Demark, and The United States (Grant et al., 2006). A noteworthy association between *TCF7L2* gene and T2DM has also been demonstrated in other studies (Damcott et al., 2006; Groves et al., 2006; Scott et al., 2006; van Vliet-Ostapchouk et al., 2007). The results of the present study (strongly for rs7903146 and rs12255372) were in consistent with all the aforementioned studies by Zhang et al.'s on Caucasians, Lyssenko et al.'s on Scandinavians, Villareal et al.'s on Americans, Muendlein et al.'s on Australians, Assmann et al.'s on Southern-Brazilians, and Grant et al.'s on Icelandic, Danish, and American subjects.

Four *TCF7L2* gene variants (rs11196205, rs7901695, rs12255372, and rs7903146) were investigated within a Japanese sample. A significant association was found between T2DM and these variants. Among of all studied SNPs, rs12255372 showed the strongest association (Hayashi et al., 2007). Jia et al., conducted a hospital-based case-control study to evaluate the relationship between the *TCF7L2* gene variant (rs7903146) and T2DM risk in a Chinese population. They concluded that rs7903146 is independently associated with the risk of developing T2DM under co-dominant, dominant, and recessive models of inheritance (Jia et al., 2016). Kaya et al., evaluated six intronic SNPs of *TCF7L2* among 169 individuals with T2DM and 119 healthy controls which were separately associated with T2DM. Their results indicated that, in the Turkish population, the T allele of the rs7903146 (C > T)

and rs12255372 (G > T) polymorphisms of *TCF7L2* is an independent risk factor for the development of T2DM (Erkoc Kaya et al., 2017). In a study, Cropano et al., explored the mechanism by which the risk allele of rs7903146 confers susceptibility to impaired glucose tolerance (IGT) or T2DM in obese adolescents. In this study, rs7903146 was genotyped in a multiethnic cohort of 955 youths. The results showed that rs7903146 risk allele was associated with higher 2-h glucose levels in Caucasians ($P = 0.006$) and African-Americans ($P = 0.009$), and a trend was also seen in Hispanics ($P = 0.072$). Likewise, the T allele was associated with decreased β -cell responsively and IGT ($P < 0.05$). Suppression of endogenous hepatic glucose production was lower in subjects with the risk variant ($P = 0.006$). Lastly, the odds of showing IGT/T2DM at follow-ups, were higher in subjects carrying the minor allele ($P = 0.0012$) (Cropano et al., 2017). In another study, three SNPs of *TCF7L2* including rs12255372, rs11196205, and rs7901695 were investigated with T2DM risk among 163 individuals from a Turkish population. This proved that rs12255372 is associated with T2DM (Vural, 2017). Chang et al., carried out a meta-analysis aimed to systematically evaluate *TCF7L2* gene variants with gestational diabetes mellitus (GDM) susceptibility in all population and racial/ethnic subgroups to come up with a foundation for future research. Stratified analysis based on race/ethnicity was also carried out. A total of 22 studies were covered, capturing eight *TCF7L2* SNPs and involving 5573 cases and 13,266 controls. Six of eight SNPs showed significant association with GDM occurrence, of which within them, rs7903146, rs12255372 and rs7901695 were the most powerful SNPs. Stratified analysis by race/ethnicity showed discrepant results in these three SNPs. In Caucasians and other races, all these SNPs were found to have a significant association with GDM risk, but in Asians, only SNP rs7903146 showed a significant association. Six of eight SNPs were found to have significant associations between *TCF7L2* variants and GDM risk in the overall population, with the most powerful SNPs being rs7903146, rs12255372 and rs7901695. (Chang et al., 2017). Haddad et al., conducted a study on 69 genes involved in Wnt pathway which showed that *TCF7L2* was the only gene significantly associated with T2DM ($P < 0.01$). One of the three studied variants of *TCF7L2*, rs114770437, was not associated with the GWAS index SNP rs7903146 and may signify an independent association signal observed only in African ancestry populations (Haddad et al., 2017). Noordam et al., investigated the association of rs7903146 with measures of glucose metabolism and measures of adiposity. This cross-sectional analysis was performed in 5744 middle-aged participants from the Netherlands Epidemiology of Obesity (NEO) Study. Their study results showed that rs7903146-T is associated with a decreased insulin concentration and increased risk of T2DM with opposing effects of adjustment for adiposity (Noordam et al., 2018). Ho et al., investigated the association of *TCF7L2* gene variants with GDM susceptibility via a meta-analysis through a total of 19 eligible case-control articles. A significantly increased GDM risk was observed for rs7903146 (all OR > 1, $P < 0.01$), but not for rs12255372. (Hou et al., 2017).

Shokouhi et al., studied the association of rs7903146, rs12255372,

Table 4
Clinical characteristics of non-T2DM and T2DM individuals based on all genotypes of TCF7L2 gene variants (rs7903146, rs12255372, and rs11196205).

SNP	Group	Genotype	Gender F/M	P	Age (years)	P	BMI (kg/m ²)	P	FBS (mmol/l)	P	SBP (mm Hg)	P	DBP (mm Hg)	P	HbA1c (mmol/mol (%))	P	Age of onset (years)	P
rs7903146	Individual with T2D	CC	122/33	0.24	52.91 ± 9.3	0.91	28.77 ± 5.12	0.35	8.45 ± 3.37	0.79	131.6 ± 23	0.06	76.9 ± 10	0.58	57 ± 20 (7.4 ± 1.8)	0.03	44.3 ± 9.6	0.78
		CT	80/61		52.97 ± 9.44		28.6 ± 5.32		8.74 ± 3.75		127.9 ± 19		76.1 ± 8.9		57 ± 16 (7.4 ± 1.5)		45.1 ± 10.1	
		TT	94/40		52.54 ± 9.5		27.94 ± 4.2		8.56 ± 3.8		126.3 ± 19.6		75.7 ± 9.5		68 ± 21 (8.4 ± 1.9)		45.2 ± 16.14	
Individual without T2D	CC	106/81	0.05	55.93 ± 11.7	0.03	26.63 ± 4.3	0.73	5.13 ± 0.33	0.90	124.6 ± 18.3	1E-3	77.8 ± 9.4	0.02	32 ± 13 (5.1 ± 1.2)	0.37	-	-	-
	CT	114/59		58.6 ± 13.1		26.4 ± 4.1		5.14 ± 0.38		127.4 ± 17		78.0 ± 9.2		32 ± 16 (5.1 ± 1.5)		-	-	-
	TT	30/30		54.2 ± 11.9		26.9 ± 3.5		5.12 ± 0.33		117.5 ± 12.2		74.3 ± 7.2		33 ± 20 (5.2 ± 1.8)		-	-	-
rs11196205	Individual with T2D	GG	211/72	0.84	53.5 ± 9.8	0.21	28.24 ± 5.2	0.51	8.33 ± 3.2	0.14	129.0 ± 21.2	0.47	76.1 ± 9.5	0.62	57 ± 20 (7.4 ± 1.8)	0.03	45.26 ± 10.24	0.68
		GC	154/53		51.95 ± 9.1		28.81 ± 4.68		9.04 ± 4.2		127.3 ± 9.35		76.6 ± 9.2		57 ± 16 (7.4 ± 1.5)		44.35 ± 14.03	
		CC	37/10		52.9 ± 9.2		28.4 ± 4.95		8.52 ± 3.45		131.0 ± 20.95		75.1 ± 8.8		68 ± 21 (8.4 ± 1.9)		45.56 ± 9.63	
Individual without T2D	GG	139/91	1.00	57.25 ± 12.48	0.67	26.51 ± 3.4	0.94	5.14 ± 0.37	0.30	125.0 ± 18.9	0.21	77.4 ± 9.2	0.32	32 ± 13 (5.1 ± 1.2)	0.37	-	-	-
	GC	103/68		56.55 ± 12.12		26.65 ± 4.37		5.15 ± 0.32		125.7 ± 15.1		78.1 ± 9.3		32 ± 16 (5.1 ± 1.5)		-	-	-
	CC	25/16		55.45 ± 12.7		26.5 ± 3.97		5.05 ± 0.38		119.8 ± 15.1		75.5 ± 8.3		33 ± 20 (5.2 ± 1.8)		-	-	-
rs12255372	Individual with T2D	GG	115/35	0.84	53.38 ± 8.96	0.39	29.1 ± 5.4	0.16	8.34 ± 3.31	0.40	130.3 ± 20.95	0.32	76.4 ± 8.7	0.79	32 ± 13 (5.1 ± 1.2)	0.37	44.87 ± 9.74	0.17
		GT	201/70		53.02 ± 9.64		28.1 ± 4.8		8.6 ± 3.6		127.2 ± 19.2		75.9 ± 9.5		32 ± 16 (5.1 ± 1.5)		45.71 ± 13.24	
		TT	80/28		51.77 ± 9.9		28.5 ± 4.7		9 ± 4.16		129.6 ± 22.7		76.6 ± 9.3		33 ± 20 (5.2 ± 1.8)		43.09 ± 10.6	
Individual without T2D	GG	121/91	0.26	56.2 ± 11.4	0.54	26.7 ± 4.36	0.80	5.13 ± 0.33	0.99	124.0 ± 18	0.23	77.2 ± 9.2	0.57	57 ± 20 (7.4 ± 1.8)	0.03	-	-	-
	GT	117/63		57.6 ± 13.34		26.6 ± 4.1		5.14 ± 0.38		126.5 ± 16.8		78 ± 9.5		57 ± 16 (7.4 ± 1.5)		-	-	-
	TT	28/21		56.83 ± 12.88		26.1 ± 3.2		5.13 ± 0.35		121.7 ± 14.5		76.7 ± 7.5		68 ± 21 (8.4 ± 1.9)		-	-	-

Data are shown as means ± SD (frequency in %), P as p-value indicates independent t-test and Fisher's exact test, BMI: Body Mass Index, F.B.S.: Fasting blood sugar, SBP: Systolic blood pressure, DBP: Diastolic blood pressure, and F/M: Female/Male.

and rs290487 with T2DM among 173 patients with T2DM and 173 healthy subjects included in Iranian Kurdish ethnic group. In their research, frequencies of T-allele among cases were significantly higher than control subjects; T-allele of rs12255372, rs7903146, and rs290487 conferred susceptibility to T2DM (Shokouhi et al., 2014). Comparing the present study with the Shokouhi et al.'s study, this study surveyed 537 T2DM patient and 437 control subject population of northern Iran. The current study genotyped the three *TCF7L2* variants (rs7903146, rs11196205 and rs11255372) in the study subject by MGB Taqman assay. This study analyzed single locus, LD and multi-locus haplotype analysis using SNPalyze (ver. 8.5 Dinacon, Japan). However another study by Pourahmadi et al., had reported the association between *TCF7L2* variant with T2DM and insulin resistance in the southern part of Iran; however, in their paper, rs12255372 and rs7903146 were not associated with insulin resistance in the evaluated population. Also, in some studies performed in China, India, and the United Arab Emirates no significant association was found between *TCF7L2* variants and T2DM (Chang, Chang et al., 2007, Guo et al., 2007, Saadi et al., 2008). It is noticeable that the current findings and previous research, differ in the selection of patients and controls in various studies and population stratifications based on gene pool differences between various geographic areas of Iran as well as other countries. Furthermore, our previous study on the association of *ENPP1* with T2DM revealed remarkable impacts of *ENPP1* variants on the incidence of T2DM among northern Iranians (Sharafshah et al., 2018). This study had some limitations such as demographic factors specifically ethnicity, race, and diet warrant consideration.

In conclusion, this is the first study performed in the north ethnic group from Iran, in which a significance of genetic variations of the *TCF7L2* was investigated in the pathogenesis of T2DM. The findings of the current study revealed that rs7903146 and rs12255372 and haplotype analysis embedded (rs7903146, rs11196205 and rs11255372) of *TCF7L2* were associated with the risk of T2DM in the north of Iran.

Conflict of interest

The authors of the present study have no conflict of interest to disclose.

Acknowledgments

We are sincerely grateful and have special thanks for the consultation and help of Dr. B. Soltani in Cellular and Molecular Research Center, Guilan University of Medical Sciences, Rasht, Iran.

References

Alberti, K.G.M.M., Zimmet, P.f., 1998. Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: diagnosis and classification of diabetes mellitus. Provisional report of a WHO consultation. *Diabet. Med.* 15 (7), 539–553.

Assmann, T.S., Duarte, G.C., Rheinheimer, J., Cruz, L.A., Canani, L.H., Crispim, D., 2014. The *TCF7L2* rs7903146 (C/T) polymorphism is associated with risk to type 2 diabetes mellitus in Southern-Brazil. *Arquivos Brasileiros de Endocrinologia & Metabologia* 58 (9), 918–925.

Chang, Y.-C., Chang, T.-J., Jiang, Y.-D., Kuo, S.-S., Lee, K.-C., Chiu, K.C., Chuang, L.-M., 2007. Association study of the genetic polymorphisms of the transcription factor 7-like 2 (*TCF7L2*) gene and type 2 diabetes in the Chinese population. *Diabetes* 56 (10), 2631–2637.

Chang, S., Wang, Z., Wu, L., Lu, X., Shanguan, S., Xin, Y., Li, L., Wang, L., 2017. Association between *TCF7L2* polymorphisms and gestational diabetes mellitus: a meta-analysis. *Journal of Diabetes Investigation* 8 (4), 560–570.

Cropano, C., Santoro, N., Groop, L., Dalla Man, C., Cobelli, C., Galderisi, A., Kursawe, R., Pierpont, B., Goffredo, M., Caprio, S., 2017. The rs7903146 variant in the *TCF7L2* gene increases the risk of prediabetes/type 2 diabetes in obese adolescents by impairing β -cell function and hepatic insulin sensitivity. *Diabetes Care* 40 (8), 1082–1089.

Damcott, C.M., Pollin, T.I., Reinhart, L.J., Ott, S.H., Shen, H., Silver, K.D., Mitchell, B.D., Shuldiner, A.R., 2006. Polymorphisms in the transcription factor 7-like 2 (*TCF7L2*) gene are associated with type 2 diabetes in the Amish: replication and evidence for a role in both insulin secretion and insulin resistance. *Diabetes* 55 (9), 2654–2659.

Erkoc Kaya, D., ARIKOĞLU, H., KAYIŞ, S.A., ÖZTÜRK, O., GÖNEN, M.S., 2017.

Transcription factor 7-like 2 (*TCF7L2*) gene polymorphisms are strong predictors of type 2 diabetes among nonobese diabetics in the Turkish population. *Turkish J. Med. Sci.* 47 (1).

Florez, J.C., Jablonski, K.A., Bayley, N., Pollin, T.I., de Bakker, P.I., Shuldiner, A.R., Knowler, W.C., Nathan, D.M., Altshuler, D., 2006. *TCF7L2* polymorphisms and progression to diabetes in the Diabetes Prevention Program. *N. Engl. J. Med.* 355 (3), 241–250.

Grant, S.F., Thorleifsson, G., Reynisdottir, I., Benediktsson, R., Manolescu, A., Sainz, J., Helgason, A., Stefansson, H., Emilsson, V., Helgadóttir, A., 2006. Variant of transcription factor 7-like 2 (*TCF7L2*) gene confers risk of type 2 diabetes. *Nat. Genet.* 38 (3), 320.

Groves, C.J., Zeggini, E., Minton, J., Frayling, T.M., Weedon, M.N., Rayner, N.W., Hitman, G.A., Walker, M., Wiltshire, S., Hattersley, A.T., 2006. Association analysis of 6,736 UK subjects provides replication and confirms *TCF7L2* as a type 2 diabetes susceptibility gene with a substantial effect on individual risk. *Diabetes* 55 (9), 2640–2644.

Guo, T., Hanson, R.L., Traurig, M., Muller, Y.L., Ma, L., Mack, J., Kobes, S., Knowler, W.C., Bogardus, C., Baier, L.J., 2007. *TCF7L2* is not a major susceptibility gene for type 2 diabetes in Pima Indians: analysis of 3,501 individuals. *Diabetes* 56 (12), 3082–3088.

Haddad, S.A., Palmer, J.R., Lunetta, K.L., Ng, M.C., Ruiz-Narváez, E.A., Consortium, M., 2017. A novel *TCF7L2* type 2 diabetes SNP identified from fine mapping in African American women. *PLoS One* 12 (3), e0172577.

Hayashi, T., Iwamoto, Y., Kaku, K., Hirose, H., Maeda, S., 2007. Replication study for the association of *TCF7L2* with susceptibility to type 2 diabetes in a Japanese population. *Diabetologia* 50 (5), 980–984.

Hou, Z., Li, M., Cao, Y., 2017. *TCF7L2*, *CAPN10* polymorphisms are associated with gestational diabetes mellitus (GDM) risks: a meta-analysis. *Gynecol. Endocrinol.* 33 (5), 399–404.

Jia, H., Li, Q., Lv, L., 2016. Association between transcription factor 7-like 2 genetic polymorphisms and development of type 2 diabetes in a Chinese population. *Genet. Mol. Res.* 15 (2).

Karnes, J.H., Gong, Y., Pacanowski, M.A., McDonough, C.W., Arwood, M.J., Langaee, T.Y., Pepine, C.J., Johnson, J.A., Cooper-DeHoff, R.M., 2013. Impact of *TCF7L2* single nucleotide polymorphisms on hydrochlorothiazide-induced diabetes. *Pharmacogenet. Genomics* 23 (12), 697.

Keshavarz, P., Habibi-pour, R., Ghasemi, M., Kazemnezhad, E., Alizadeh, M., Omami, M.H.H., 2014. Lack of genetic susceptibility of *KCNJ11* E23K polymorphism with risk of type 2 diabetes in an Iranian population. *Endocr. Res.* 39 (3), 120–125.

Körner, A., Berndt, J., Stumvoll, M., Kiess, W., Kovacs, P., 2007. *TCF7L2* gene polymorphisms confer an increased risk for early impairment of glucose metabolism and increased height in obese children. *J. Clin. Endocrinol. Metab.* 92 (5), 1956–1960.

Lyssenko, V., Lupi, R., Marchetti, P., Del Guerra, S., Orho-Melandar, M., Almgren, P., Sjögren, M., Ling, C., Eriksson, K.-F., Mancarella, R., 2007. Mechanisms by which common variants in the *TCF7L2* gene increase risk of type 2 diabetes. *J. Clin. Invest.* 117 (8), 2155–2163.

Muendlein, A., Saely, C.H., Geller-Rhomberg, S., Sonderegger, G., Rein, P., Winder, T., Beer, S., Vombank, A., Drexler, H., 2011. Single nucleotide polymorphisms of *TCF7L2* are linked to diabetic coronary atherosclerosis. *PLoS One* 6 (3), e17978.

Noordam, R., Zwetsloot, C., de Mutsert, R., Mook-Kanamori, D., Lamb, H., de Roos, A., de Koning, E., Rosendaal, F., van Dijk, K.W., van Heemst, D., 2018. Interrelationship of the rs7903146 *TCF7L2* gene variant with measures of glucose metabolism and adiposity: the NEO study. *Nutr. Metab. Cardiovasc. Dis.* 28 (2), 150–157.

Ouhaibi-Djellouli, H., Mediène-Benchekor, S., Lardjam-Hetraf, S.A., Hamani-Medjaoui, I., Meroufel, D.N., Boulououar, H., Hermant, X., Saidi-Mehtar, N., Amouyel, P., Houti, L., 2014. The *TCF7L2* rs7903146 polymorphism, dietary intakes and type 2 diabetes risk in an Algerian population. *BMC Genet.* 15 (1), 134.

Saadi, H., Nagelkerke, N., Carruthers, S.G., Benedict, S., Abdulkhalek, S., Reed, R., Lukic, M., Nicholls, M.G., 2008. Association of *TCF7L2* polymorphism with diabetes mellitus, metabolic syndrome, and markers of beta cell function and insulin resistance in a population-based sample of Emirati subjects. *Diabetes Res. Clin. Pract.* 80 (3), 392–398.

Safarpour, M., Ebrahimi, A., Daneshpour, M.S., 2015. From genome to gene: a review of genes and genetic variations associated with type 2 diabetes. *Tehran University Medical Journal* 73 (9), 615–623.

Saxena, R., Gianniny, L., Burt, N.P., Lyssenko, V., Giuducchi, C., Sjögren, M., Florez, J.C., Almgren, P., Isomaa, B., Orho-Melandar, M., 2006. Common single nucleotide polymorphisms in *TCF7L2* are reproducibly associated with type 2 diabetes and reduce the insulin response to glucose in nondiabetic individuals. *Diabetes* 55 (10), 2890–2895.

Scott, L.J., Bonnycastle, L.L., Willer, C.J., Sprau, A.G., Jackson, A.U., Narisu, N., Duren, W.L., Chines, P.S., Stringham, H.M., Erdos, M.R., 2006. Association of transcription factor 7-like 2 (*TCF7L2*) variants with type 2 diabetes in a Finnish sample. *Diabetes* 55 (9), 2649–2653.

Sharafshah, A., Keshavarz, P., Rezaei, S., Farhadian, N.J.G., 2018. Association and in silico studies of *ENPP1* gene variants with type 2 diabetes mellitus in a Northern Iranian population. 675, 225–232.

Shokouhi, S., Delpisheh, A., Haghani, K., Mahdizadeh, M., Bakhtiyari, S., 2014. Association of rs7903146, rs12255372, and rs290487 polymorphisms in *TCF7L2* gene with type 2 diabetes in an Iranian Kurdish ethnic group. *Clin. Lab.* 60 (8), 1269–1276.

Tsai, M.-K., Wang, H.-M.D., Shiang, J.-C., Chen, I., Wang, C.-C., Shiao, Y.-F., Liu, W.-S., Lin, T.-J., Chen, T.-M., Chen, Y.-H., 2014. Sequence variants of *ADIPOQ* and association with type 2 diabetes mellitus in Taiwan Chinese Han population. *Sci. World J.* 2014.

van Vliet-Ostapchouk, J., Shiri-Sverdlov, R., Zhernakova, A., Strengman, E., Van Haefen, T., Hofker, M., Wijmenga, C., 2007. Association of variants of transcription

- factor 7-like 2 (TCF7L2) with susceptibility to type 2 diabetes in the Dutch Breda cohort. *Diabetologia* 50 (1), 59–62.
- Vijan, S., 2010. Type 2 diabetes. *Ann. Intern. Med.* 152 (5), ITC3–1-1.
- Villareal, D.T., Robertson, H., Bell, G.I., Patterson, B.W., Tran, H., Wice, B., Polonsky, K.S., 2010. TCF7L2 variant rs7903146 affects the risk of type 2 diabetes by modulating incretin action. *diabetes* 59 (2), 479–485.
- Vural, H.C., 2017. Tcf7l2 gene polymorphism in T2dm with patients in Turkish population. *Journal of Clinical Epigenetics* 3 (3), 27.
- Yan, Y., 2009. Diabetes Susceptibility Polymorphisms and Risk of Prediabetes and Diabetes Complications in the Atherosclerosis Risk in Communities (ARIC) Study. The University of North Carolina at Chapel Hill.
- Yang, Y., Xu, J., Wang, Y., Liu, X., 2015. Association of TCF7L2 gene polymorphisms with susceptibility to type 2 diabetes mellitus in a Chinese Hui population. *Genet. Mol. Res.* 14, 10064–10071.
- Zhang, C., Qi, L., Hunter, D.J., Meigs, J.B., Manson, J.E., van Dam, R.M., Hu, F.B., 2006. Variant of transcription factor 7-like 2 (TCF7L2) gene and the risk of type 2 diabetes in large cohorts of US women and men. *Diabetes* 55 (9), 2645–2648.