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Evaluation of Transcription Factor 7 like 2 polymorphisms and haplotypes in risk of Type 2 Diabetes

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Abstract

Type 2 diabetes (T2D) is a chronic disorder with different genetics and environmental factors. It is one of growing diseases in the world. Previous studies show association between Transcription Factor 7 Like2 (TCF7L2) and T2D. The current study set to evaluate the relation between TCF7L2 polymorphisms and T2D in Southeast Iran. The present case-control study was done on 250 T2D and 250 healthy controls (HCs). For genotyping polymorphisms TCF7L2 (rs11196205) and (rs4132670) Amplification-Refractory Mutation System-Polymers Chain Reaction (ARMS-PCR) was used. The results showed frequency rates of GC and CC genotypes increased in patients compared to controls (31% vs. 6% and 55% vs. 8%, respectively), showing a statistically significant difference (OR=2.67(1.37-5.21), P<0.05 and OR=3.31(1.92-5.71), P< 0.05, respectively). The C allele was associated with an increased risk of T2D, with the frequency of 28% and 11% in patients and controls, respectively (OR=3.11 (2.22-4.37), P< 0.05). Another Polymorphism of this gene TCF7L2 (rs4132670) was not associated with T2D. Furthermore, the haplotype analysis revealed that rs11196205C/ rs4132670C and rs11196205C/ rs4132670T are risk factors against T2D (OR=2.08 (1.49-2.86, P<0.05 and OR=1.72 (1.06-2.78) P<0.05, respectively). The findings demonstrated that TCF7L2 (rs11196205) genotypes GC, CC, and allele (C) confer risk for susceptibility to T2D.

Keywords: *Gene polymorphism; Haplotype; Hyperglycemia; TCF7L2.*

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Introduction

Type 2 diabetes (T2D) is a heterogeneous and multi-factorial metabolic disease (genetic and environmental factors), characterized by increase in peripheral blood glucose (hyperglycemia) due to insulin resistance (decreased action of insulin at targeted tissues) and/or pancreatic beta-cell dysfunction (1). T2D is known as non-insulin dependent diabetes, accounting for 90% of diabetic patients who possess several pathogenic factors such as lifestyle, overeating, and genetic elements, implying a 2.4 fold increased risk for T2D in individuals with positive family history (2). To manage their hyperglycemia and protect themselves against long-term complications of the disease such as retinopathy, nephropathy, neuropathy, and cardiovascular problems (3), patients with T2D must use drugs such as 1.oral hypoglycemic agents, oral sulfonylureas 1st generation (like Chlorpropamide, Tolazamide, and Tolbutamide), oral sulfonylureas 2nd generation

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(like Glimepiride, Glipizide, Glyburide, and Gliclazide), non-sulfonylurea secretagogues (like Nateglinide and Repaglinide), biguanides (like Metformin), Thiazolidinediones (like Pioglitazone and Rosiglitazone), and alpha-glucosidase inhibitors (like Acarbose) 2. Novel targets for increased insulin action include glucagon-like peptide-1 (GLP-1), dipeptidyl peptidase IV (DDP-4) inhibitors, glucagon receptor antagonists, and sodium-glucose co-transporter 2 (SGLT2) inhibitors (2, 4).

The prevalence of diabetes in Eastern Mediterranean and West Asian regions including Iran was 9.3% in 2010 and will reach 10.8% in 2030, and as regards the worldwide prevalence, diabetes among adults is predicted to rise to approximately 439 million patients in 2030, and in developing countries such as Iran, this prevalence will be much greater than in developed countries (5).

Genes have important roles in pathogenesis of T2D. Transcription Factor 7 Like 2 (TCF7L2) (also known as Transcription Factor 4) is located on chromosome 10q25.3 and consists of 17 exons (6); this gene is expressed in various tissues, especially tissues involved in the pathogenesis of T2D including fat, liver, and pancreatic islets of Langerhans. It acts as transcription factor (TF) in the Wnt-signaling pathway. This signaling pathway has an important role in embryogenesis consisting of adipocyte and pancreatic tissue formation (7). Previous studies show that TCF7L2 has a key role in insulin secretion and synthesis, which is also a key regulator of pro-insulin processing and production (8).

Some variants of TCF7L2 gene reduce insulin secretion and increase the risk of T2D (6). Several studies indicate TCF7L2 polymorphisms' association with T2D (9). Accordingly, the current inquiry set to evaluate two Single Nucleotide Polymorphisms (SNPs) (rs11196205 and rs4132670) in TCF7L2 gene in a case-control study on Southeast Iran.

Materials and methods

A case-control study was performed on 250 participants with T2D in Ali-Asghar Hospital of Zahedan, Southeast Iran (Sep, 2015). All the individuals were confirmed as T2D patients through biomedical tests such as Fast Blood Sugar (FBS) (Enzymatic Colorimetric Assay, Pars-Azmoon Co, Tehran, Iran) on Autoanalyzer BT 1500 (Biotecnica, Italy) more or equal to 126 mg/dl, Hemoglobin A1c (HbA1c) (NycoCardTM HbA1c on NycoCard Reader II (Axis-Shield, Oslo, Norway)) more or equal to 6.7%. Although more or equal to 6.5% of HbA1c is international guideline for diagnose of T2D, normal range in our region (Southeast of Iran) taking into account gender, age, and ethnicity is 6.7% and were considered by endocrinologists in diagnosing all T2D patients.

In total, 250 healthy controls (HCs) were enrolled on a voluntary basis without any systemic disease and relation with T2D from Razmjo-Moghadam, Zahedan, Southeast Iran, and they had normal biomedical data (inclusion criteria for HCs were normal FBS and/or HgA1C, FBS≤100 or HbA1c<6.7). The convenient sampling method was used for both groups. All T2D and HCs participants were Iranian and provided their informed consents before taking part in the study. The ethics committee of Zahedan University of Medical Sciences approved the protocol of the study.

Genotype analysis

The salting out phenol chloroform method was used for extraction of genomic DNA from 2 ml peripheral blood leukocyte (stored at -20°C until genotyping). The primers were designed based on ARMS method (**Table 1**). The final PCR mixture volume was 20 µl consisting of ~80-100 ng genomic DNA, 10 pM of each primer (forward and reverse), 10 µl master mix, and 7 µl distilled water. Bio-Rad thermal cycler (Bio-Rad, Hercules, CA, USA) was used

Table 1. Allele-Specific Polymerase (ASP), Polymerase Chain Reaction Primers sequences

Primer 5'-3'	Product	Method
Rs11196205	211bp	ARMS
Fw;CCATAACTCTCTTACATACTGCCG		
Fm:CCATAACTCTCTTACATACTGCCC		
R:TGCTACTGTGCATGATCCTG		
Rs4132670	256bp	ARMS
Rw:GTGCAGCCCAGACATAGCAGAG		
Rm:GTGCAGCCCAGACATAGCAGAA		
F:AGAGTTGCATCGTATGCCAA		

for amplification with the following condition: rs11196205, an initial denaturation at 95°C for 6 min followed by 30 cycles at 95°C for 30 sec, annealing 55°C for 30 sec, extension at 72°C for 30 sec, and after the completion of the thirtieth cycle, the final extension was at 72°C for 6 min. The rs4132670 condition was similar to previous SNP (rs11196205), except that the annealing temperature was 58°C.

PCR products were verified on Agarose 2% containing ethidium bromide (0.6 μg/ml), observed with gel electrophoresis (under UV light). The sizes of PCR products were 211 base pair (bp) for rs11196205 and 256bp for rs4132670. Some samples, either T2D or HCs, were re-genotyped randomly; the present results confirmed the past findings.

Statistical analysis

SPSS 16.0 (SPSS Ine., Chicago, USA) was used for data analysis. Logistic regression was used to find the association between SNPs and T2D and compute 95% confidence interval (95% CI) and the Odd Ratio (OR). Independent samples t-test and χ^2 test were used for analyzing clinical and demographic data. The significance levels for all tests were considered $P \leq 0.05$. The frequency of haplotypes was calculated using PHASE version-2.1 software (19, 20). Haplotypes with estimated frequency <5% were excluded from the analysis. Multivariate logistic regression models were used to evaluate the associations between T2D and TCF7L2 genotypes.

Results

Subjects' characteristics

The clinical and demographic features of the T2D patients and HCs are shown in **Table 2**. No significant difference was found regarding age, gender, triglyceride (TG), and low-density lipoprotein (LDL) between two groups ($P>0.05$). There were statistically significant differences in total cholesterol (TC) ($P=0.02$), and high-density lipoprotein (HDL) ($P=0.002$) between the groups; furthermore, the level of FBS, HbA1c, and Body Mass Index (BMI) were significantly higher in the T2D group ($P<0.0001$).

Genotyping of TCF7L2 SNPs

The two SNPs of TCF7L2 gene were successfully genotyped in 250 T2Ds and 250 HCs. The frequency rates of alleles and genotypes of TCF7L2 polymorphisms are shown in **Table 3**. All the loci did not conform to the Hardy-Weinberg equilibrium (HWE) in T2D and HCs ($P<0.05$). In T2D group, the genotype distribution of both SNPs, rs11196205G/C and rs4132670C/T, was not in HWE ($X^2=120.34$, $P<0.05$ and $X^2=122.14$, $P<0.05$, respectively), and in HCs, the genotype distribution of rs11196205G/C ($X^2=129.05$,

Table 2. Demographic characteristics of T2D patients and controls

	T2D (n ± SD)	Controls (n ± SD)	P-value
Age (year)	54.87 ± 10.13	48.86 ± 10.01	0.593
Sex(Female/male)	183/67	174/76	0.429
FBS (mg/dl)	188.40 ± 86.45	97.70 ± 19.21	<0.0001
TC (mg/dl)	183 ± 44.31	181.84 ± 36.1	0.020
TG (mg/dl)	161.40 ± 83.24	148.1 ± 94.59	0.792
HDL (mg/dl)	55.4 ± 20.17	54.01 ± 14.68	0.002
LDL (mg/dl)	97.21 ± 34.33	104.49 ± 29.06	0.355
BMI (kg/m2)	27.63 ± 5.49	21.48 ± 2.43	<0.0001
HbA1c	9.21 ± 1.78	4.93 ± 0.94	<0.0001

FBS = Fast blood sugar; TC = Total Cholesterol; TG = Triglyceride; HDL = High-dense lipoprotein; LDL = Low-dense lipoprotein; BMI = Body Mass Index; HbA1c = Hemoglobin A1c.

P<0.05) and rs4132670C/T (X^2=145.88, P<0.05) was not in HWE. In rs11196205G/C of TCF7L2 gene, the frequencies of GG, GC, and CC genotypes were 66%, 12%, and 22% in T2D, and 86%, 6%, and 8% in HCs, respectively. GC genotype of this SNP (rs11196205G/C) was significantly different between T2Ds and HCs (GC vs. GG, OR=2.67, 95%CI=1.27-5.21, P=0.004) as risk factor, and CC genotype was also strongly associated with T2D as risk factor (CC vs. GG, OR=3.31, 95%CI=1.92-5.71, P<0.0001). The frequencies of alleles of rs11196205G/C were

Table 3. Genotype and allele frequency of *TCF7L2 (rs11196205 and rs4132670)* polymorphisms in T2D and controls

	T2D n(%)	Controls n(%)	P-value	OR (95% CI)
TCF7L2(rs11196205)				
GG, n (%)	164(66%)	215(86%)		Ref=1.00
GC, n (%)	31(12%)	14(6%)	0.004	2.67(1.37-5.21)
CC, n (%)	55(22%)	21(8%)	<0.0001	3.31(1.92-5.71)
Allele				
G, n (%)	359(72%)	444(89%)		1.00
C, n (%)	141(28%)	56(11%)	<0.0001	3.11(2.22-4.37)
TCF7L2(rs4132670)				
CC, n (%)	133(53%)	127(51%)		1.00
CT, n (%)	36 (14%)	29(12%)	0.427	1.25(0.72-2.15)
TT, n (%)	81(33%)	94(38%)	0.447	0.86(0.59-1.26)
Allele				
C, n (%)	302(60%)	283(57%)		1.00
T, n (%)	198(40%)	217(43%)	0.247	0.85(0.66-1.10)

CI = confidence interval; OR = odds ratio.

Table 4. Single nucleotide Polymorphism association with T2D and Control

	T2D	CONTROL	P-Value	OR(95%CI)
TCF7L2(rs11196205)				
Dominant				
GG	164(66%)	215(56%)		REF=1
GC+CC	86(34%)	35(14%)	<0.0001	3.23(2.08-5)
Recessive				
GG+GC	195(78%)	229(91.6%)		1
CC	55(22%)	21(8.4%)	<0.0001	3.03(1.85-5.26)
TCF7L2(rs4132670)				
Dominant				
CC	133(53%)	127(51%)		1
CT-TT	117(47%)	123(49%)	0.59	0.91(0.64-1.28)
Recessive				
CC+CT	169(68%)	156(62%)		1
TT	81(32%)	94(38%)	0.22	0.81(0.55-1.15)

72% for G allele and 28% for C allele in T2D. On the other hand, the frequencies of G and C alleles were 89% and 11% in HCs, respectively. The C allele in rs11196205G/C was strongly associated with T2D (C vs. G, OR=3.11, 95%CI=2.22-4.37, P<0.0001) as risk factor.

In rs4132670C/T of TCF7L2 gene, the frequency rates of CC, CT, and TT genotypes in T2Ds were 53%, 14%, and 33%, and 51%, 12%, and 38% in HCs, respectively. Both genotypes, CT and TT of this SNP (rs4132670C/T), were not significantly different between T2Ds and HCs (P>0.05). There was no association between T allele of rs4132670C/T and T2D (P>0.05), while the frequencies of C and T alleles were 60% and 40% in T2Ds, and 57% and 43% in HCs, respectively.

The dominant and recessive statuses were studied (**Table 4**) for either rs11196205 or rs4132670. The results showed that in rs-11196205G/C, GC+CC was associated with T2D in dominant status (GC+CC vs. GG, OR=3.23, 95%CI=2.08-5, P<0.0001), and CC was associated with T2D in recessive status (CC vs. GG+GC, OR=3.03, 95% CI=1.85-5.26, P<0.0001). There was no significant association regarding T2D in both statuses (dominant and recessive) in rs4132670.

The frequency rates of four common haplotypes of two SNPs in TCF7L2 gene (rs11196205 and rs4132670) are shown in **Table 5**. Although there was no significant difference between GT haplotype and T2D (P=0.93), CC and CT haplo-

Table 5. Haplotype association with T2D

Rs11196205	Rs4132670	T2D	Control	P-value	OR(95%CI)
G	C	0.3956	0.4928	-	Ref=1
G	T	0.3224	0.3952	0.93	0.99(0.8-1.23)
C	C	0.2084	0.0732	<0.0001	2.08(1.49-2.86)
C	T	0.0736	0.0388	0.026	1.72(1.06-2.78)

types were significantly different between T2D and HCs, hence the risk of T2D increased to 2.08 fold in the presence of CC haplotype (OR=2.08, 95% CI=1.49-2.86) and increased to 1.72 fold in the presence of CT haplotype (OR=1.72, 95% CI=1.06-2.78, P=0.026). Finally, multivariate logistic regression was used to adjust for potential confounding factors including gender, age, FBS, TC, TG, HDL, and LDL for both SNPs (**Table 6**), indicating that CC vs. GG genotypes of rs1196205 was associated with T2D (OR=3.98, 95%CI=1.81-8.77, P=0.001), while GC vs. GG was not associated with the risk/protection of T2D (P>0.05) in this SNP. Considering rs4132670 genotype, there was no statistically significant difference among the groups.

Discussion

Type 2 diabetes, as epidemic disease, is strongly associated with TCF7L2 and also complications and risk factors such as cardiovascular diseases and obesity (6, 10). This gene is a member of TCF/lymphoid enhancer-binding factor of transcription factors involved in Wnt-signaling (11).

In the current study, two SNPs of TCF7L2 in T2D were evaluated. The results showed that GC and CC genotypes of rs11196205 of TCF7L2 gene were strongly associated with T2D as risk factor, and C allele of this gene SNP (rs4132670) was not associated with T2D. However, previous studies have proved that rs7903146C/T polymorphism of TCF7L2 is associated with T2D in different ethnic populations (12-15). There is little data regarding the role of TCF7L2 polymorphism (rs11196205 and rs4132670), especially rs4132670 polymorphism. Luo et al. found that, in meta-analysis of the association between T2D and SNPs of TCF7L2 in East-Asian population, rs11196205 was significantly associated with T2D (16). In another investigation in Hong-Kong, MCY Ng et al. showed that rs11196205 was associated with T2D as risk factor (17). Tangjittipokin et al., similarly, found that GC in Thai patients was associated with T2D, with OR equal to

Table 6. Multivariate logistic regression analysis of the relationship between *TCF7L2* polymorphisms in T2D*

Genotype rs11196205	P-Value	Odds ratio (95% CI)	Genotype rs4132670	P-Value	Odds ratio (95% CI)
GG		ref	CC		ref
GC	0.132	2.28(0.78-6.67)	CT	0.402	1.456(0.605-3.509)
CC	0.001	3.98(1.81-8.77)	TT	0.796	0.919(0.484-1.745)
Sex	0.901	0.958(0.489-1.876)	Sex	0.840	1.070(0.560-2.041)
Age	0.001	1.06(1.02-1.09)	Age	0.001	1.050(1.020-1.081)
FBS	<0.0001	1.05(1.04-1.07)	FBS	<0.0001	1.052(1.040-1.066)
TC	0.824	1.002(0.988-1.015)	TC	0.760	1.002(0.989-1.015)
TG	0.785	1.001(0.997-1.004)	TG	0.758	1.001(0.997-1.004)
HDL	0.254	1.013(0.991-1.035)	HDL	0.184	1.014(0.993-1.036)
LDL	0.181	0.990(0.974-1.047)	LDL	0.196	0.990(0.976-1.005)

**Multivariate logistic regression was used to adjust for potential confounding factors including sex, age, FBS, TC, TG, HDL, and LDL. FBS: Fast blood sugar, TC: Total Cholesterol, TG: Triglyceride, HDL; High-density lipoprotein, LDL: Low-density lipoprotein.*

1.83 (18). This SNP (rs11196205) of TCF7L2 was strongly associated with T2D in European-derived population, Pima Indians, and Chinese (19-22). In contrast, this SNP was investigated in South Sweden, revealing that neither GC genotype nor CC genotype was associated with T2D (9). There is little data on the association between rs4132670 and T2D. Pang et al. found that rs4132670 may play a role in gene expression of TCF7L2 associated with T2D (23). Another investigation was done by Karnes et al. on hydrochlorothiazide-induced diabetes, revealing that rs4132670 was associated with their study population (24). The present study had some limitations such as calorie intake, sedentary life style, and other environmental confounding factors and some other characteristics such as ethnicity, life style, and socioeconomic status of the population.

The deviation from HWE is not clear in the current study population; it may have several reasons such as small sample size, migration, or consanguineous marriages that are common in this area of the country (Zahedan, southeast Iran).

The results indicated that rs11196205 of TCF7L2 gene was associated with T2D, so C allele of this SNP is a risk factor for this disease; however, another SNP (rs4132670) of TCF7L2 gene was not associated with T2D in the study population. CC haplotype was strongly associated with T2D as risk factor, while CT haplotype was slightly associated with T2D.

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Conflict of Interests

The authors declare that there is no conflict of interests to disclose.

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