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

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Background/aim: Type 2 diabetes (T2D) is a multifactorial disease, determined by environmental and genetic factors. Currently, the transcription factor 7-like 2 (*TCF7L2*) gene shows the strongest association with T2D. In this study, we investigated whether *TCF7L2* gene polymorphisms are associated with T2D in a Turkish population.

Materials and methods: Using PCR-RFLP and PCR-SSCP, we genotyped six intronic polymorphisms in the *TCF7L2* gene, commonly associated with T2D, in 169 individuals with diabetes and 119 healthy controls.

Results: We found that rs7903146 C → T substitution in intron 3 (OR: 1.9, P = 0.005) and rs12255372 G → T substitution in intron 4 (OR: 2.1, P = 0.002) were significantly associated with T2D while other SNPs were not associated (P > 0.05). We determined no association between *TCF7L2* gene polymorphisms and fasting glucose, fasting insulin, HbA1c, or HOMA-IR levels (P > 0.05), except for rs7903146 C → T substitution, which was significantly associated with the fasting glucose level (P = 0.003).

Conclusion: Our results indicate that, in the Turkish population, the T allele of the rs7903146 (C → T) and rs12255372 (G → T) polymorphisms in the *TCF7L2* gene is an independent risk factor for the development of T2D.

Key words: Single nucleotide polymorphism, *TCF7L2* gene, type 2 diabetes mellitus, Wnt pathway

1. Introduction

Type 2 diabetes mellitus (T2DM) is one of the most challenging health problems of the 21st century. Today more than 371 million people have diabetes globally (1). This figure shows that the number of cases of diabetes is more than expected and has already reached the estimation for the year 2030 (2). Additionally, approximately 50% of people with diabetes are unaware that they have the disease. In 2012, diabetes was responsible for 4.8 million deaths, half of them under the age of 60, and 471 billion USD spent (1). The Turkish population is no exception to this trend. Understanding the mechanisms that contribute to the pathogenesis of T2DM is crucial in implementing rational treatment strategies and to prevent the increasing occurrence of diabetes. T2DM is a polygenic metabolic disorder that can occur in different age groups as a result of the interaction of genetic and environmental factors (3). To determine the genetic basis of T2DM, several genes that were predicted to be T2DM risk factors were studied in numerous populations. Contrary to expectations, many of them conferred only modest effects regarding disease risk and mostly with conflicting results (4,5). The *TCF7L2* gene (also known as *TCF-4*) on chromosome 10q25 has the strongest association with an increased risk of T2DM, and this finding has been well-replicated in multiple populations (6–21). Reports of strong associations between *TCF7L2* gene variants, especially rs7903146 T, and T2DM have been predominantly obtained from European populations. However, unlike populations of European origin, in Asian populations, either these variants are unrelated or different variants within the *TCF7L2* gene are associated with disease risk (21–25). In Turkey, which is a geographic and cultural bridge between Europe and Asia, a study that investigates the association between *TCF7L2* polymorphisms and T2DM is lacking. In this study, we aimed to determine whether *TCF7L2* variants are major contributors to T2DM in Turkish populations. Our population comprised nonobese individuals to

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expose the genetic background of T2DM more accurately by excluding obesity, an important risk factor in disease development.

2. Materials and methods

2.1. Clinical samples

Our study population included 169 unrelated nonobese patients with type 2 diabetes as the diabetic group and 119 individuals without a family history of T2DM and a body mass index (BMI) matched with the case group as the healthy control group recruited from the sample pool indicated in our previous study (26). To exclude obesity, individuals with a BMI of <30 were included in the study. Informed written consent was obtained from each individual prior to participation in the study sample pool. This study was approved by the ethics committee of Selçuk University.

2.2. Clinical analysis

Fasting plasma glucose, fasting insulin, HbA1c, and c-peptide values of the diabetic and the healthy individuals in our sample pool were measured. Our clinical criteria for inclusion in the groups and the HOMA-IR determination formula were detailed previously (26).

2.3. DNA analysis and genotyping

Genomic DNA isolation from peripheral blood leukocytes was performed using a standard proteinase K and SDS procedure. The nucleotide sequence of the *TCF7L2* gene was obtained from the GenBank database (accession no. NT_030059.13). Six intronic SNPs that are commonly associated with T2DM in the literature were genotyped. Four of the intronic SNPs in the *TCF7L2* gene were evaluated by PCR-RFLP, and two were evaluated by PCR-SSCP (polymerase chain reaction/single-strand conformation polymorphism). Six specific primers were designed for the target SNPs of the *TCF7L2* gene using an online program (www.idtdna.com). For PCR amplification, 1X PCR buffer, 0.4 mM of each primer, 0.6 mM deoxynucleoside triphosphates, 0.1 U of Taq polymerase, and 50–100 ng of genomic DNA was used in a volume of 15 µL. PCR reactions were carried out in a thermocycler (Bio-Rad, Hercules, CA, USA) with the following steps: initial denaturation at 94 °C for 5 min, followed by 35 cycles of denaturation at 94 °C for 30 s, annealing of each primer for 30 s at different temperatures, and elongation at 72 °C for 30 s, and finally extension at 72 °C for 2 min.

Following amplification, the samples to be evaluated by PCR-SSCP were diluted 5-fold with formamide buffer (80% formamide, 0.1% bromophenol blue, 0.1% xylene cyanol, 10 mM EDTA), denaturated at 95 °C for 10 min, and chilled on ice prior to electrophoresis. Samples were run on electrophoresis at 2–10 W for 15–18 h at room temperature in 10X Tris-borate-EDTA (TBE) buffer and banding was performed by silver staining. Samples were grouped according to migration profiles on the SSCP gel, and differences were analyzed by nucleotide sequencing.

Following amplification, the products of all four SNPs to be evaluated by PCR-RFLP (rs7903146, rs12255372, rs11196213, and rs3814573) were digested with the *Rsa*I, *Tas*I, *Bsr*I, and *Cac8*I restriction enzymes, respectively. Following digestion, DNA fragments were visualized by electrophoresis on a 3% agarose gel and stained with ethidium bromide. Genotyping results were validated by direct sequencing of randomly selected samples for each SNP. Sequence traces were evaluated individually, and alignments were compared to sequences available in GenBank using the NCBI blast program. Observed concordance between genotyping assays was 100%.

2.4. Statistical analysis

Descriptive statistics for clinical and biochemical properties were detailed in our previous study (26). A t-test was used for performing initial comparison between patient and control groups. Hardy–Weinberg equilibrium in patient and control groups was evaluated by performing a chi-square goodness-of-fit test. Dominant, additive, and recessive modeling was used for analyses. Dominance was defined in terms of allele 2 (minor allele) effects. In dominant allele 2 models, homozygous individuals for allele 1 were compared with carriers of allele 2. In recessive allele 2 models, homozygous individuals for allele 2 were compared with carriers of allele 1. Odds ratios (ORs) were used to evaluate allele frequencies of SNPs in patient and control groups. Additionally, patient and control group ORs were obtained for pairwise SNP genotypes to evaluate the SNP allele–type 2 diabetes associations under dominant, additive, and recessive models. Before analysis, variables of fasting plasma insulin and HOMA-IR were skewed and were normalized using log and square root transformations, respectively.

Secondly, SNP genotypes were coded as 0, 1, and 2 for genotypes 11, 12, and 22, respectively. In the subsequent stage, transformed fasting plasma insulin and HOMA-IR were analyzed by fitting single-point (single SNP) and two-point (two SNPs) regression analysis models. Fasting glucose levels of the individuals were classified as <100, 100–125, 126–200, and >200 mg/dL. To evaluate the fasting glucose level–SNP genotype relationship, the chi-square test was used. All statistical analyses were performed using the R 2.11.1 program (27). Linkage disequilibrium (LD) and haplotype frequency analysis were performed with Arlequin software. In all analyses, $P < 0.05$ was considered statistically significant.

3. Results

3.1. Clinical and biochemical properties of study subjects

The clinical and biochemical properties of the individuals included in our study were previously presented (26). As seen there, levels of fasting glucose, fasting insulin, HbA1c, c-peptide, and HOMA-IR in patients with T2DM were significantly different compared to the control group ($P < 0.05$). There was no significant difference for BMI between diabetic (26.7 ± 8.2) and control (26 ± 8.4) groups, as expected ($P = 0.20$).

3.2. Association study

All SNPs were in Hardy–Weinberg equilibrium in our population ($P > 0.05$). No association was observed between the rs7901695 T → C, rs11196205 G → C, 11196213 C → T, and rs3814573 C → T substitutions and T2DM according to chi-square and odds calculations ($P > 0.05$). The rs7903146 C → T substitution in intron 3 was associated under dominant (OR: 1.71 [95% CI: 1.06–2.77], $P = 0.01$) and additive (OR: 1.9 [95% CI: 1.15–3.19], $P = 0.005$) models, and the rs12255372 G → T substitution in intron 4 was significantly associated under dominant (OR: 2.1 [95% CI: 1.25–3.55], $P = 0.002$) and additive (OR: 1.99 [95% CI: 1.14–3.48], $P = 0.007$) models with T2DM (Table 1). The T allele was determined to be a diabetic risk allele for both of the SNPs. Calculated odds ratios were higher compared to those in the current literature. Remarkably, for all studied SNPs, heterozygote and homozygote rare allele frequencies were higher than common alleles, compared to other populations. Genotype distributions are presented in Table 1. LD was calculated among the eight SNPs and r^2 values did not provide evidence of pairwise LD between the SNPs (Table 2). We conducted haplotype frequency analysis using the six SNPs. Fifty-seven haplotypes in the diabetic group and 42 haplotypes in the control group were observed in our population. The most common haplotypes were TTTCTT (16.66%) in the diabetic group and CGCCTT (15.7%) in the control group for rs7903146, rs12255372, rs7901695, rs11196205, rs11196213, and rs3814573, respectively. The most common haplotype, TTTCTT, in the patient group was observed at only 0.8% in the control group.

Table 1. Genotype distribution of SNPs in the *TCF7L2* gene and association analysis with type 2 diabetes.

	Gene region	SNP		Genotype n (%)		Odds ratio (95% CI-P)		
						Additive	Dominant	Recessive
1	(intron 3)	rs7901695 Diabetic Control	T/T 50 (29.76) 27 (24.11)	C/T 82 (48.80) 62 (55.33)	C/C 36 (21.43) 23 (20.53)	1.40 (0.62–3.16) 0.205	1.3 (0.6–2.8) 0.24	1.11 (0.57–2.16) 0.37
2	(intron 3)	rs7903146 Diabetic Control	C/C 58 (34.52) 57 (47.5)	C/T 95 (54.76) 47 (39.16)	T/T 18 (10.71) 16 (13.33)	1.9 (1.15–3.19) 0.005	1.71 (1.06–2.77) 0.01	0.78 (0.38–1.6) 0.25
3	(intron 4)	rs11196205 Diabetic Control	G/G 18 (11.25) 16 (14.68)	G/C 89 (55.62) 54 (49.54)	C/C 53 (33.12) 39 (35.77)	0.81 (0.41–1.61) 0.27	0.91 (0.47–1.74) 0.39	0.63 (0.26–1.5) 0.15
4	(intron 4)	rs12255372 Diabetic Control	G/G 72 (42.85) 30 (27.5)	G/T 65 (38.69) 51 (46.78)	T/T 31 (18.45) 28 (25.68)	1.99 (1.14–3.48) 0.007	2.1 (1.25–3.55) 0.002	1.63 (0.9–2.94) 0.055
5	(intron 4)	rs11196213 Diabetic Control	C/C 50 (35.21) 22 (20.95)	C/T 73 (51.40) 69 (65.71)	T/T 19 (13.38) 14 (13.3)	2.06 (0.95–4.44) 0.07	1.98 (0.94–4.19) 0.08	0.98 (0.38–2.48) 0.48
6	(intron 4)	rs3814573 Diabetic Control	C/C 59 (38.31) 51 (46.36)	C/T 67 (43.5) 43 (39.09)	T/T 28 (18.18) 16 (14.54)	0.78 (0.39–1.56) 0.24	0.75 (0.39–1.44) 0.2	1.26 (0.51–3.12) 0.3

Table 2. Standardized pairwise LD coefficients D' and r2 of SNPs of *TCF7L2* gene.

SNP		D' →					
		rs7903146	rs12255372	rs7901695	rs11196205	rs11196213	rs3814573
r2 ↓	rs7903146	*	0.5017	0.3250	0.5598	0.4334	0.1889
	rs12255372	0.2133	*	0.1420	0.3419	0.3711	0.1834
	rs7901695	0.0798	0.0129	*	0.3154	0.2723	0.2546
	rs11196205	0.1329	0.0420	0.0558	*	0.5554	0.4258
	rs11196213	0.1720	0.1275	0.0513	0.1197	*	0.2358
	rs3814573	0.0357	0.0285	0.0769	0.0769	0.0509	*

D' is shown above diagonal of empty cells.
r2 is shown below diagonal of empty cells.

3.3. Genotype–phenotype association

The rs7903146 C → T substitution was significantly associated with fasting glucose level (P = 0.003), while the other substitutions displayed no association (P > 0.05). HOMA-IR, HbA1c, and c-peptide values were statistically evaluated for fasting insulin levels and no association was observed between the SNPs and these phenotypic values (P > 0.05). Additionally, SNP–SNP interactions and the combined effects of the SNPs on T2DM, fasting insulin, fasting glucose, and HOMA-IR values were evaluated using a two-point regression model, but no significant interaction was found.

4. Discussion

T2DM has a strong genetic basis; however, prior to 2006, this was insufficiently explained by most of the candidate genes (3,4). In 2006, Grant et al. (6) reported that *TCF7L2* gene variants were strongly associated with an increased T2DM risk in various case-control subjects from Iceland, Denmark, and the United States. This study attracted considerable worldwide attention, and similar association results have been consistently replicated in several subsequent studies from different ethnic groups (7–21). *TCF7L2* encodes a transcription factor involved in the Wnt signaling pathway, which critically influences endocrine pancreatic development and modulates mature β-cell functions including insulin secretion, survival, and proliferation (28), and it acts as a nuclear receptor for β-catenin (29). The results of five large-scale genome-wide association studies (19,30–33) and data obtained from comprehensive metaanalysis studies (11,34,35) revealed that *TCF7L2* is the most important (in terms of susceptibility) T2DM gene among those identified to date. One SNP in particular, rs7903146 T, has been found to be associated with type 2 diabetes in several populations, and it is regarded as one of the strongest genetic risk factors for the development of T2DM (12).

All of the *TCF7L2* variants that are associated with T2DM are intronic substitutions. rs7903146 C → T and rs7901695 T → C in intron 3, and rs12255372 G → T and rs11196205 G → C in intron 4, are the most notable SNPs in *TCF7L2*. The strong linkage between these four SNPs has been shown in multiple studies (35). In the present study, we aimed to explore the effects of these four SNPs and additionally rs1196213 and rs3814573 in a Turkish population.

In our study population, the rs7903146 C → T substitution in intron 3 and the rs12255372 G → T substitution in intron 4 are strongly associated with T2DM, which is consistent with the majority of the current literature. The odds ratios (1.9 and 2.1) were higher compared to those in the literature; however, they were similar to the odds ratios calculated for rs7903146 and rs12255372 (1.9 and 1.8, respectively) in the metaanalysis study in which the data from 36 different populations were evaluated (35). Our results suggest that the T allele of both the rs7903146 and rs12255372 substitutions is an independent risk factor for white Europeans as well as West African, Mexican, African American, Indian, and Japanese populations.

We did not observe an association between the rs7901695 T → C, rs11196205 G → C, 11196213 C → T, and rs3814573 C → T substitutions and T2DM (P > 0.05). According to LD analysis, we did not detect any significant linkage between the SNPs evaluated in this study. Furthermore, our findings suggest that the TTTCTT haplotype for rs7903146, rs12255372, rs7901695, rs11196205, rs11196213, and rs3814573 is an important risk indicator for the development of T2DM in Turkish populations.

An interesting finding was that the risk allele frequencies of the four SNPs rs7901695, rs11196205, 11196213, and rs3814573 were higher in East Asian populations and lower in Caucasian populations, consistent with the findings in our Caucasian population. The frequencies of genetic variants in humans, including *TCF7L2*, vary between populations. Evidence suggests that a high frequency of variants at genetic loci included in metabolism in some populations occurred as a consequence of natural selection (36). In particular, the observed rs7903146 T SNP frequency is highly variable at a wide range in the world. The rs7903146 T variant is represented at less than 5% in most East Asian and Native American populations, while it has a frequency nearing 50% in African populations as shown by Guinan (37).

Guinan's epidemiology study demonstrated extensive global and regional variations in the frequency of *TCF7L2* SNPs, the effects of which may impose a contrasting risk of disease in different regions and populations of the world and may contribute to differences in the incidence of T2DM globally. The diverse geographic distribution of *TCF7L2* SNPs is likely because of genetic diversity believed to be shaped by the effect of a range of factors such as the impact of historical founder effects, migration, population admixture, and genetic drift occurring throughout human evolution and affecting the distribution of *TCF7L2* SNPs globally (37).

In the literature, common allele frequencies of the SNPs studied in our work were strikingly higher compared to the incidence in heterozygous and homozygous rare alleles in general. In our study, both in the diabetic and control groups, rare and especially heterozygous allele frequencies were found to be higher than those of common alleles. We consider that this finding is due to the genetic admixture of Anatolia that results from it being the cradle of several civilizations throughout the history of mankind and its geographical position that lies in the center of migration roads, existing as a bridge between Europe and Asia (38). Therefore, the frequency of the heterozygote genotype may show differences when compared to communities without genetic admixture such as Northern European, Scandinavian, and Native American communities, where genetic stratification is known to occur.

Notwithstanding the established effect of *TCF7L2* variants on T2DM risk, the downstream molecular effects of these SNPs and how the variants confer susceptibility to T2DM remain unclear. Functional studies have suggested various mechanisms such as an impairment in insulin secretion by glucose-induced insulin secretion or incretin-induced insulin secretion, development of insulin resistance, or affected proinsulin conversion to insulin.

Damcott et al. (7) and Chandak et al. (13) suggested that *TCF7L2* variants are associated with increased insulin resistance. However, in the majority of the studies, these gene variants were associated with reduced insulin secretion rather than reduced insulin activity (6,11,39–43). Although the association between decreased insulin secretion and *TCF7L2* is not yet fully understood, it has been hypothesized that in particular the presence of the rs7903146 T allele constitutes a T2DM risk by reducing glucose-induced insulin secretion (41) or by reducing incretin-induced insulin secretion, which is secreted from enteroendocrine cells (6,39). Alternatively, because *TCF7L2* has a key role in Wnt signaling, which is critical for the development of the pancreas and islets during embryonic growth, it may cause impairment of β-cell mass, pancreatic β-cell development, and/or β-cell function (44).

In the present study, only the rs7903146 C → T substitution was significantly associated with fasting glucose levels ($P = 0.003$), while other substitutions were not associated ($P > 0.05$). Fasting insulin, HOMA-IR, HbA1c, and c-peptide values were not associated with the SNPs ($P > 0.05$). The lack of association in our study between HOMA-IR and the variants with T2DM in addition to a strong statistical association between the rs7903146 C > T substitution and the fasting glucose level further suggest that *TCF7L2* risk variants affect the development of T2DM by influencing the secretion of insulin from the pancreas rather than insulin resistance. Taken together with the data from existing studies, it is observed that *TCF7L2* variants contribute to a decrease in insulin secretion and impairment of incretin hormone GLP-1 activity, which stimulates pancreatic β-cell output.

Identification of genes and associated variants related to type 2 diabetes is important to explain the pathophysiology of the disease, to detect individuals under risk of disease development at an early stage by developing models to be used in disease risk estimation, to determine drug–genome interactions, and, taking this total information together, to develop diagnostic, preventive, and therapeutic methods for clinical management. The *TCF7L2* gene is quite promising in terms of meeting the expectations for clinical benefit among those identified to date. This study provides the initial data from a Turkish population regarding the association between the *TCF7L2* gene and T2DM development among nonobese diabetics. Our results support an association between the *TCF7L2* gene and the disease and indicate that the rs12255372 and rs7903146 risk alleles are independent risk factors for T2DM development in this Turkish population and may be useful for clinical management of the disease.

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