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Research Article

Association of the *TCF7L2* rs12255372 (G/T) variant with type 2 diabetes mellitus in an Iranian population

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Abstract

In various populations worldwide, common variants of the *TCF7L2* (Transcription factor 7-like 2) gene are associated with the risk of type 2 diabetes mellitus (T2DM). The aim was to investigate the association between rs12255372 (G/T) polymorphism in the *TCF7L2* gene and T2DM in an Iranian population. 236 unrelated patients with T2DM, and 255 normoglycemic controls without diabetes were studied. The PCR-RFLP method was used for genotyping rs12255372 (G/T) polymorphism, and the SPSS version 18.0 for Windows for statistical analysis. The minor T allele of *TCF7L2* rs12255372 was found to significantly increase the risk of T2DM, with an allelic odds ratio (OR) of 1.458 (95% CI 1.108-1.918, p = 0.007). A significant difference in TT genotype was observed between T2DM patients and normoglycemic controls (OR 2.038, 95% CI 1.147-3.623; p = 0.014). On assuming dominant and recessive models, ORs of 1.52 [95% CI (1.05-2.21) p = 0.026)] and 1.74 [95% CI (1.01-3.00) p = 0.043] were obtained, respectively, thereby implying that the co-dominant model would best fit the susceptible gene effect. This study further confirms the *TCF7L2* gene as enhancing susceptibility to the development of T2DM.

Key words: TCF7L2 gene, rs12255372 variant, type 2 diabetes mellitus (T2DM), single nucleotide polymorphism (SNP).

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Introduction

Type 2 diabetes mellitus (T2DM) is a heterogeneous metabolic disorder arising from the interplay of genetic and environmental factors (Barroso, 2005). Diabetes and its associated complications pose a major health-care burden worldwide, presenting major challenges to patients well-being, health-care systems and national economies (Ramachandran *et al.*, 2010). Although the prevalence of T2DM worldwide is about 6%, it is likely to rise over the next decade, due to the increasing age of the population, and surge of obesity (Zimmet, *et al.*, 2001). Accounting for 90~95% of populations with diabetes, T2DM is predicted to reach 300 million people worldwide by the year 2025 (Zimmet *et al.*, 2001; Grant *et al.*, 2006;). There are significant geographic and racial differences in incidence, this ranging from 4.6 to 40% in the Middle East (Al-Nuaim, 1997; Abdella *et al.*, 1998; Husseini *et al.*, 2000; Al-Habori *et al.*, 2004), 1.2% to 14.6% in Asia, and 1.3% to 14.5% in Iran (Azizi *et al.*, 2003a,b).

The transcription factor 7–like 2 gene (TCF7L2), also known as TCF-4, is a nuclear receptor for CTNNB1 (previously known as β-catenin), which in turn mediates the canonical WNT signaling pathway (Polakis, 2000; Smith, 2007). Recent genome-wide association studies have dramatically increased knowledge on the genetic background of T2DM. Intronic variants of the *TCF7L2* gene, besides having been set forth as possible genetic risk factors for T2DM, have also been associated with alterations in the function of beta cells among normoglycemic individuals (Bonetti *et al.*, 2011). Current evidence presupposes that the predominant effect of *TCF7L2* dysfunction on type 2 diabetes development may be mediated through the impair-

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ment of insulin secretion (Damcott *et al.*, 2006; Florez *et al.*, 2006; Saxena *et al.*, 2006; Scott *et al.*, 2006; Chandak *et al.*, 2007). Grant and colleagues have reported the association of a common microsatellite (DG10S478) within intron 3 of *TCF7L2* with T2DM. They showed that allele G of SNP rs12255372 is in strong linkage disequilibrium (LD) with allele 0 of DG10S478, as allele T of rs12255372 is with other alleles of DG10S478 (Grant *et al.*, 2006).

There are at least four well-studied polymorphic markers in the human*TCF7L2* gene, which are associated with T2DM, viz., rs7903146, rs7901695, rs12255372 and rs11196205. Most published epidemiological studies have placed emphasis on rs7903146 (C/T) and rs12255372 (G/T) variations (Luo *et al.*, 2009; Tong *et al.*, 2009), with the consequential association of rs7903146 *TCF7L2* polymorphism with T2DM (Amoli *et al.*, 2010).

In this study, the aim was to investigate the association between the *TCF7L2* rs12255372 variant and T2DM in an Iranian population. To our knowledge, this SNP has not been previously tested in the Iranian population.

Subjects and Method

Subjects

The studied population comprised 236 unrelated T2DM patients and 255 normoglycemic controls without diabetes. The T2DM patients were recruited from the Gorgan Clinic of Diabetes, Golestan University of Medical Sciences, north Iran. The normoglycemic controls, recruited from the same area, were age-matched with the case population. T2DM-patient diagnosis, based on the standard criteria (American Diabetes Association, 2004), with fasting plasma glucose ≥ 126 mg/dL, 2-h plasma glucose ≥ 200 mg/dL during an oral glucose tolerance test. Written informed consent was obtained from each subject. Patients with the age-of-onset < 37 years were excluded. All those selected for this study were of Fars origin.

Clinical characteristics

Anthropometric measurements of weight, height and waist-circumference were obtained by standardized techniques. The body mass index (BMI) was calculated as the weight in kilograms divided by the square of the height in meters. HbA1c levels were defined by using turbidometric inhibition immunoassaying (Tina Quant, Roche, Basel, Switzerland).

BMI, waist circumference and HbA1c were measured in T2DM patients and normoglycemic controls.

DNA extraction and genotyping

Genomic DNA was extracted from peripheral blood leukocytes using the phenol/chloroform procedure. The study was approved by the Ethics committee of Golestan University of Medical Sciences (No: 1090). The rs12255372 polymorphism was genotyped using the PCR-RFLP method. Briefly, the region was amplified with the following primers: Forward 5'- CTG GAA ACT AAG GCG TGA GG -3'; Reverse 5'- GGG TCG ATG TTG TTG AGC TT -3'. The PCR cycles were as follows: 5 min at 94 °C, followed by 35 cycles of 30 s at 94 °C, 30 s at 54 °C and 30 s at 72 °C. Final extension was for 10 min at 72 °C. Subsequently, 10 μL of the 346 bp amplified product was digested with the *Tsa*I (Tsp509I) restriction enzyme (Fermentas, EU) for 3 h at 65 °C. After treatment with restriction enzymes, amplicons were subjected to 3.5% agarose gel electrophoresis and stained with ethidium bromide. The studied region usually has two restriction sites, the G → T allele change creating a new one. Some genotyping results were validated by direct sequencing.

Statistical analysis

Data analysis was with SPSS Statistical software (version 18, SPSS, Chicago, IL, USA). A P value of < 0.05 was considered as significant. Mean ± SD and relative frequency were used for quantitative and qualitative traits, respectively. The SHAPIRO-WILK test was applied to diabetic and normoglycemic subjects, to compare normality of quantitative traits in both groups, the mean of these traits being subsequently compared by t-testing. The association of SNPs with T2DM in matched case-control subjects was tested using χ^2 analysis, as well as the calculation of Odds ratios (ORs), with 95% confidence intervals (CIs).

Results

A case-control association study was undertaken, comprising 236 T2DM patients and 255 normoglycemic controls, all over 37 years-of-age. 35 subjects (20 normoglycemic controls and 15 T2DM patients) were excluded, through unavailability of individual genomic DNA, thus leaving 221 T2DM patients and 235 normoglycemic controls for genotyping. Genotype frequencies did not differ from the expected Hardy-Weinberg ratios.

The minor T allele of the *TCF7L2* rs12255372 was found to significantly increase T2DM risk, with an allelic odds ratio (OR) of 1.458 (95% CI 1.108-1.918, p = 0.007) in the population under study. T allele frequency was 38.7% in T2DM patients and 30.2% in normoglycemic controls (Table 1).

A comparison of genotype frequencies in T2DM patients and normoglycemic controls showed a significant difference in the frequency of the TT genotype (OR 2.038, 95% CI 1.147-3.623; p = 0.014), although not so in the GG and GT genotypes (Table 1).

To investigate which model would fit the effect of *TCF7L2* rs12255372, dominant and recessive models were considered. In the dominant model (GG *vs.* TT and GT), the genotype frequencies were compared between the T2DM and control groups and a significant association with T2DM was observed (OR 1.52, 95% CI (1.05-2.21)

p = 0.026). Assuming the recessive model (GG and GT *vs.* TT), a significant association was also found (OR 1.74, 95% CI (1.01-3.00) p = 0.043). Thus, the highest risk was observed under the co-dominant model (OR 2.038, 95% CI (1.147-3.623) p = 0.014) (Table 1).

Based on rs12255372 (GT) genotypes, the clinical and biochemical characteristics of the T2DM patients and normoglycemic individuals were stratified. With the exception of waist circumference, which was significantly higher in G allele homozygotes (p = 0.002), no association was found between this variant and either age, BMI or HbA1c in T2DM patients (Table 2). Comparative analyses of normoglycemic controls and rs12255372 (G/T) genotypes also revealed no association of clinical characteristics with the genotype (data not shown).

Discussion

In this study, the aim was to explore the association of *TCF7L2* rs12255372 polymorphism with Type 2 diabetes (T2DM) in an Iranian population. We found that the minor T allele of *TCF7L2* rs12255372 significantly increases T2DM risk, with an allelic odds ratio (OR) of 1.458 (95% CI 1.108-1.918, p = 0.007) (Table 1). Although the strongest association with T2DM risk in most reported studies is with rs7903146, some studies have shown a stronger association with rs12255372 (Scott *et al.*, 2006; Chandak *et al.*, 2007; Hayashi *et al.*, 2007; Wang *et al.*, 2007; Saadi *et al.*, 2008).

However, no association between a *TCF7L2* rs12255372 variant and T2DM has been reported in Chinese, Arabic and Pima Indian populations (Chang *et al.*, 2007; Guo *et al.*, 2007; Alsmadi *et al.*, 2008;). Furthermore, a modest association of *TCF7L2* rs12255372 with T2DM was shown in an African-American population (Sale *et al.*, 2007). The underlying mechanisms, by which genetic variation within the intron of the *TCF7L2* gene confers susceptibility to type 2 diabetes, remain to be elucidated. Genetic variations near the 3' end of the *TCF7L2* gene may affect the action of *TCF7L2*, through the regulation of alternative splicing (Chang *et al.*, 2007).

Frequency of the minor T allele in the normoglycemic controls was found to be 30.2%, thus, although consistent with Palestinian, Amish and French populations (29.3, 29, and 30%, respectively) (Cauchi *et al.*, 2006; Damcott *et al.*, 2006; Ereqat *et al.*, 2010), quite different from that reported in a Chinese population (3%) (Ren *et al.*, 2008). The frequency of the TT genotype carrying the two minor alleles (risk alleles) was significantly different in T2DM patients compared to normoglycemic controls, thereby implying a possible gene dosage effect. However, although the frequency of the GT genotype was higher in the T2DM patients compared to normoglycemic controls, no significant difference was observed between GT genotypes and T2DM.

To determine whether there is any interaction between rs12255372 (G/T) genotypes and clinical and biochemical characteristics, the association between genotypes and covariates in T2DM patients and normoglycemic controls was studied separately. Although no association was found between the variant and age, BMI and HbA1c in T2DM patients, in an earlier study of Emirati subjects (Saadi *et al.*, 2008), waist circumference was found to be significantly higher in G allele homozygotes.

The study proved the rs12255372 variant of the *TCF7L2* gene to be associated with T2DM in the Iranian population, in contrast with previous reports on Chinese,

Table 1 - The *TCF7L2* rs12255372 polymorphism allele and genotype frequencies in T2DM patients and normoglycemic controls.

Genotype	normoglycemic controls (n = 235)	T2DM * patients (n = 221)	OR (95% CI)	p value†
GG (%)	118 (50.2)	88 (39.8)	-	-
GT (%)	92 (39.1)	95 (42.9)	1.385 (0.930-2.061)	0.109
TT (%)	25 (10.6)	38 (17.1)	2.038 (1.147-3.623)	0.014
G allele (%)	328 (69.8)	271 (61.3)	-	-
T allele (%)	142 (30.2)	171 (38.7)	1.458 (1.108-1.918)	0.007

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

Table 2 - Clinical characteristics of the T2DMM subjects stratified by rs12255372 (G/T) genotypes.

Variable	GG	GT	TT	p value
Age (years)	52.40 ± 7.32	51. 29 ± 10.68	51.61 ± 11.68	0.741
Waist circumference(cm)	104.54 ± 13.29	97.87 ± 11.92	99.68 ± 10.99	**0.002**
BMI (kg/m^2)	29.82 ± 5.10	29. 15 ± 4.75	28.88 ± 5.51	0.34
HbA1C	8.75 ± 1.93	9.00 ± 2.19	8.73 ± 2.05	0.656

Arab and Pima Indian populations. According to the conclusions divulged in a recently published meta-analysis (Tong *et al.*, 2009), the magnitudes of this association are moderate. TT homozygous variants would approximately cause a 1.9-fold increase in T2MD as concluded by results of many studies, a value that is lesser but still close to our own result (OR = 2.038). A possible explanation for this incongruity could be differences in ethnic background, or the effects of environmental factors, such as life-style. Further studies are required to clarify the exact mechanism of its effect.

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