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The association of TCF7L2 Gene (rs12255372) single nucleotide Polymorphism with Type Two Diabetes Mellitus in Al Najaf Governorate

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Abstract:

Background: In Iraq, type 2 diabetes mellitus (T2DM) is increasing and accounts for a high proportion of medical costs. Worldwide studies have shown polymorphisms within the transcription factor 7-like 2 (TCF7L2) genes like (rs12255372) are associated with T2DM

Aim: To study the association of TCF7L2 gene polymorphism (rs12255372) with T2DM in AL Najaf population .

Methods: The study consisted of 60 T2DM patients and 60 healthy control individuals. Genotyping of rs12255372 polymorphism is carried out by PCR–RFLP. DNA was extracted from whole blood and genotyping was achieved with specific primers to amplify fragments for digestion with restriction enzymes. Tsp509I used for TCF7L2 gene product followed by electrophoresis on agarose gel. Various statistical analyses were applied to analyze the data.

Results: The amplification product of TCF7L2 was 346 bp. Digestion of TCF7L2 product demonstrated (143, 104 bp), two (126,104 bp) and three (104,126,143 bp) bands for those with wild type (GG), homozygous (TT) and heterozygous (GT) genotypes respectively.

The T allele frequency was significantly higher in the diabetic group (0.44) than in the control group (0.18). This allele was significantly associated to a greater risk of developing T2DM as compared to the G allele OR = 3.52 (95% CI 1,96 – 6.33, $p < 0.0001$).

Conclusion: Our findings suggest that the rs12255372 (G/T) polymorphism of the TCF7L2 gene is confer susceptibility to T2DM in the AL Najaf population.

INTRODUCTION

Diabetes mellitus (DM) is a group of metabolic diseases characterized by hyperglycemia, resulting from impairment of insulin secretion and/or action [1]. Several characteristic symptoms may be present with DM such as polyuria , polydipsia and polyphagia [2]. Diabetes mellitus is one of the most common chronic diseases in human populations. The prevalence of DM is 6.5% [3].

The current classification of DM is by American Diabetes Association of DM based on the causes of DM: **Type 1 DM** in which pancreatic beta cells may be destructed and this produce absolute insulin insufficiency, **Type 2 DM** in which resistance of insulin and / or insulin secretion defect, **Gestational DM** in which Deficiency and resistance of insulin in pregnancy and other types of DM including maturity onset diabetes of the young (MODY)[4].

Type 2 diabetes mellitus is the mostly predominant type when it is contrasted to other two main kinds of diabetes. It is accountable for 90% of the overall prevalence of diabetes [5].

Type 2 diabetes mellitus is characterized by the combination of disturbances in insulin secretion by pancreatic B cells and peripheral insulin resistance . the consequence of impaired insulin secretion and/or resistance, glucose uptake and release by essential tissues is disturbed, which eventually results in hyperglycemia[6].The etiology of type 2 diabetes mellitus appears to involve complex interactions between environmental and genetic factors[7].

The fact that type 2 diabetes is a polygenic disease (many genes involved). there are a number of genes so far identified as having an effect on developing the disease. However, the gene TCF7L2 stands out as having the greatest risk for developing type 2 diabetes. When the common variants single-nucleotide polymorphism (SNPs); rs12255372(G/T) and rs7903146(C/T). Both SNPs were shown to be associated with an increased risk of developing type 2 diabetes for those with impaired glucose tolerance.[8]. These genetic factors in combination with environmental factors, such as sedentary life style, obesity, and ethnic background contribute to an individual's chance of developing the disease [9]. The association between T2D and a number of single-nucleotide polymorphisms (SNPs) in the TCF7L2 gene has since been strongly confirmed in multiple Genome-wide association studies (GWAS) in different ethnic groups and this gene remains the most replicated and most strongly associated T2D risk gene at this time [10].

TCF7L2 gene encodes a transcription factor that plays a role in the Wnt signaling pathway, a key cell developmental and growth regulatory mechanism[11].

This study aimed to assess the association of TCF7L2 gene polymorphism (rs12255372) with T2DM in AL Najaf population .

METHODS

This Study was included 120 subjects . The patient population included 60 subjects with type II diabetes mellitus, patients age ranged between 35-60 years, they randomly selected from who attended the who attended the Diabetic Center at Al-Sadder Medical City, Najaf, Iraq during their routinely visiting periods for clinical examination and regular checking glucose level.

Inclusion criteria includes :

1. Fasting blood glucose level ≥ 126mg/dl.
2. The symptom of diabetes(polyuria, nocturia, polyphagia, weight loss).
3. Patients who diagnosed by physicians as having type 2 diabetes.

Exclusion criteria includes :

1. Patients they are ≤ 30 years old.
2. They have type 1 Diabetes or need insulin injection.
3. They have heart failure, cardiomyopathy or congenital heart disease.
4. They have an autoimmune disease (Rheumatic arthritis, cancer, infection, sever renal or liver disease, pregnant or currently using glucocorticoid, the cause of obesity is not due to disease (Cushing or hormonal disorder).
5. Smoker and alcoholic.

A 60 of apparently healthy individuals (without Diabetes mellitus)were randomly selected and included in the study with an age range 35-60 Years.

The practical part of the study was carried out in laboratory of Clinical Laboratory Sciences department / College of Pharmacy / Kufa University.

Peripheral blood samples of T2DM and control groups were collected in EDTA-anticoagulated tubes, and then DNA was extracted from whole-blood samples using the genomic DNA extraction kit (Favorgene). Then DNA concentration and purity were measured by UV absorption at 260 and 280 nm (BioDrop, U.K)

Genotyping was performed by polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP) for TCF7L2 gene using thermocycler (Biometra, Germany). The sequence of primers used was : forward 5" - CTG GAAACT AAG GCG TGA GG - 3" and reverse 5" - GGG TCG ATG TTG TTG AGC TT - 3". Amplification was performed in a total volume of 25 µl contained 12.5 µl GoTaq Green Master Mix, (Promega Corporation, Madison, WI), 1.5 µl of each primer (1 Mm final concentration) (OneAlpha, U.S.A), 3.5 µl nuclease free water, and 6 µl of DNA template. Cycling condition was 95°C for 2 min followed by 35 cycles of 94°C for 30s, 59.8°C for 30s, 72°C for 30s, and a final extension of 72°C for 4 min. The product was digested with 10u of restriction enzyme (Tsp509I) (Promega) and run on 3% agarose gel. To determine genotyping error rate, we performed random duplication in 20% of the samples.

Statistical analysis

Genotype and allele frequencies were compared using the $\chi 2$ statistics or the fisher's exact test. The Hardy Weinberg equilibrium was tested using the goodness-of-fit chi-square. Odd ratios were calculated by logistic regression . A p value less than 0.05 was considered statistically significant.

RESULTS

The PCR product ofTCF7L2 gene polymorphism (**rs12255372**) i.e, the amplicon is of 346 bp was digested by (Tsp509I) restriction enzyme. The product of digestion were analyzed by agarose gel electrophoresis. Results demonstrate (143, 104 bp), two (126,104 bp) and three (104,126,143 bp) bands for those with wild type (GG), homozygous (TT) and heterozygous (GT) genotypes respectively(figure 1) .

One hundred and twenty cases were positive for genotyping, characterized on agarose gel by two bands of 143 bp and 104 bp for the wild type homozygote GG, two bands of 126 bp and 104 bp for the mutant homozygote TT, and three bands of 143 bp, 126 bp and 104 bp for the mutant heterozygote GT. Fragments smaller than the 100 bp of the molecular weight marker were not visualized

The frequency of the GG genotype was 65.83% (79/120), versus 5.83% (7/120) and 28.33% (34/120) for the GT and the TT genotypes respectively. Genotype frequencies violated the Hardy-Weinberg equilibrium in the general population. The G allele was major with a frequency of 69%, as compared to the minor T allele which showed a frequency of 31%. The T allele frequency was 44.17% in diabetic group

against 18.33% in non diabetic and was found to significantly increase the risk of T2DM with an odds ratio of 3.52 (95% CI 1,96 – 6.33, p < 0.0001).

The frequency of the TT genotype was significantly higher in diabetics than in controls (41.66 % vs. 15 %) and was found to be significantly associated to T2DM with an OR of 4.08 (95% CI 1.68 a – 9.88, p = 0.0018)

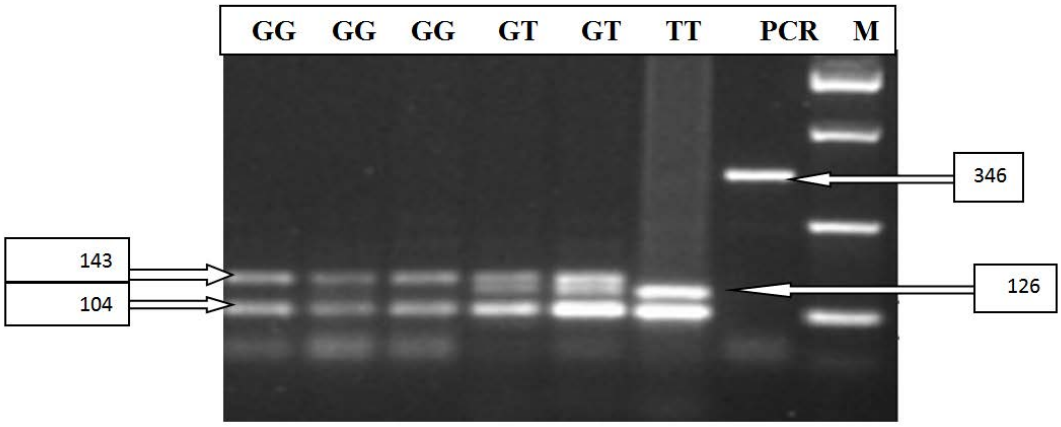

Figure 1. Results of TCF7L2 gene polymorphism(rs12255372) product on agarose gel electrophoresis.

PCR product of TCF7L2 gene was digested with Tsp509I restriction enzyme. The products of digestion were electrophoresed on 3% agarose (75V and120 min) and directly visualized with ethidium bromide under UV light.

Line 1: DNA Marker.
Lines 2 PCR product 346 bp
Lines 3 : TT genotype-126,104 bp.
Lines 4, 5: GT genotype-143, 126,104 bp
Lines 6, 7,8: GG genotype104,143 bp

Table 1 Association between the TCF7L2 rs12255372 (G/T) polymorphism and type 2 diabetes mellitus

rs12255372(G/T)	Controls, n (%)	T2DM, n (%)	OR (95% CI)	p value
Alleles				
G	98 (8a1.66)	67 (55.83)	/	/
T	22 (18.3)	53 (44.16a)	3.52 (1,96 – 6.33 a)	0.0001
Total (2 N)	120	120		
Genotypes				
GG	47 (78.33)	32 (53.33 a a)	/	/
GT	4 (6.67)	3 (5.0)	1.1 (0.23 – 5.25a)	0.9
TT	9 (15.0 a)	25 (41.66 a)	4.08 (1.68 a – 9.88)	0.0018a
Total (N)	60 a	60		
T2DM: Type 2 diabetes mellitus; OR: odd ratio				

DISCUSSION

The identification of genetic variants influencing T2DM is a major focus of research to perceive the mechanisms underlying the pathogenesis of this disorder as well as related pathological consequences. Such attempt may improve the plans of protection, diagnosis and treatment of Iraqi society. Advances such as the development of genome-wide association studies (GWAS) have enabled the identification of a number of genes associated with T2DM risk. In this scenario, common genetic variant in studies have identified TCF7L2 gene polymorphism (rs12255372) as a T2DM susceptibility locus . Thus, this SNP was investigated in the current study in Iraqi Arabic type 2 diabetic patients.

The frequency of the minor T allele was found to be 31%, and was comparable to those observed in the Czech population (30.15%) [12], the Iranian population (34.45%) [13] and the Arab population (36.15%) [14]. The variation of the T allele frequency across population could be explained by the genetic diversity between different ethnic groups [15]. This allele was found to be significantly associated to the risk of T2DM with an OR of 3.52 (95% CI 1,96 – 6.33, $p < 0.0001$).

This result is consistent with those reported by previous studies in different populations [13,16,17], where a strong association was noted between this polymorphism and the risk of T2DM. Furthermore, a weak association was reported in West-Africa, with an OR of 1.31 (95% CI 1.01–1.69, P = 0.044) [18] and in Afro-Americans [19]. However, no association between a TCF7L2 rs12255372 (G/T) variant and T2D was found in Chinese [20], Pima Indians [21], and South-African populations [22]

The frequency of the TT genotype was significantly higher in diabetic patients than normoglycemic individuals (41.66% vs. 15%, p = 0.0018). The GT genotype frequency was similar between the 2 groups and no association was found with of T2D.

CONCLUSION

1. The rs12255372 (G/T) polymorphism of the TCF7L2 gene is probably associated with T2DM in this population.
2. This variant could help to predict the occurrence of T2DM in the Al Najaf population .

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