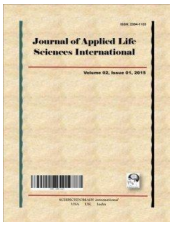

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Association of rs7903146 TCF7L2 (C/T) Gene Polymorphism and Type 2 Diabetes Mellitus in Pakistani Population

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Authors' contributions

This work was carried out in collaboration between all authors. Author MHD designed the study, Authors MHD, HJB and AML performed the statistical analysis, Author MHD wrote the protocol and wrote the first draft of the manuscript. Authors BI and HJB managed the analyses of the study. Authors MHD, BI and AML managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Among various genetic determinants of type 2 DM (T2DM), *TCF7L2* (transcription factor 7 like 2) polymorphisms are among the few verified genetic variants with large effects on the risk of T2DM in different populations. In the current study, the single nucleotide polymorphism (SNP) rs7903146 (c.382-41435C>T) was screened in 322 T2DM cases and 226 controls by genotyping using amplification refractory mutation system-polymerase chain reaction (ARMS-PCR). The *TCF7L2* SNP was found to be associated with T2DM; (odd ratio [OR] = 2.72; 95% confidence interval [CI] 2.00-3.71, P = 6.65×10^{-12}). Therefore, we report the role of *TCF7L2* variant in individuals with T2DM in Pakistani population.

Keywords: Type 2 diabetes; genetic association; transcription factor 7 like 2; Pakistan.

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1. INTRODUCTION

Diabetes Mellitus (DM) is a metabolic disease characterized by chronic hyperglycemia and disturbances in carbohydrates, lipids and proteins metabolism due to defects in insulin secretion and its action, which results in severe acute and chronic complications [1,2]. It is a chronic disease leading to various complications such as coronary heart disease, diabetic nephropathy, neuropathy and retinopathy [3]. DM is a major public health problem worldwide. Estimates from the International Diabetes Federation (IDF) indicate that there were about 381.8 million adults with diabetes mellitus in the world in 2013. This prevalence is projected to expand by 55% in 2035 to reach 591.9 million of adults affected [4,5]. Both genetic and environmental factors play a strong role in the manifestation of this complex genetic disorder [6]. Type 2 diabetes mellitus (T2DM) causes morbidity, disability and early mortality, and is associated with a huge economic burden [7]. The development of high throughput micro-array platforms unraveled a large number of genes involved in the etiology of T2DM. Among them, TCF7L2 (Transcription factor 7 like 2) is considered as one of the most important candidate genes which plays a major role in blood-glucose homeostasis and beta cell function [8]. Strong association of TCF7L2 with T2DM was initially found in Icelandic population which has been subsequently replicated in Danish and U.S populations [9]. The three TCF7L2 SNPs (rs7390146, rs12255372 and rs11196205) that were strongly associated with T2DM in the above study were subsequently replicated, along with other SNPs of TCF7L2, in a huge meta-analysis prompting their inclusion in any future replication effort [10].

The first genome wide association studies (GWAS) study on T2DM in the French population showed strong signal for TCF7L2. Its association, along with the other T2DM genes, was also confirmed in subsequent GWAS studies [9,11]. The consistency in the findings of its association with T2DM observed among many studies of diverse ethnic groups was considered indicative of a universal contribution of this gene to T2DM, although few studies showed weak or no association with T2DM, which can be attributed to the extremely low frequency of the risk alleles and inadequate sample size, hence lack of power of the study [12–14]. We present here the result of our analysis of the SNP of TCF7L2 (rs7903146) and its association with T2DM.

2. MATERIALS AND METHODS

2.1 Sample Collection

The current case-control genetic association study was in accordance with Helsinki declaration and approved by Ethical Review Board of the Department of Bioscience, COMSATS Institute of Information Technology (CIIT) Islamabad.

Whole blood samples of T2DM were collected from Mayo Hospital (Lahore), Shifa International Hospital (Islamabad), Railway Hospital (Rawalpindi) and Armed Forces Institute of Ophthalmology (Rawalpindi). All cases were clinically diagnosed for T2DM by a professional endocrinologist and had T2DM for more than 10 years. The inclusion criteria of the patients was in accordance with the American Diabetic Association (ADA) criteria for the diagnosis of T2DM i.e. age 18–75 years, fasting plasma glucose level ≥ 126 mg/dl, random plasma glucose concentration ≥ 200 mg/dl, and serum creatinine concentration ≤ 2.0 mg/dl. The controls for the study were sampled from the same general population (Pakistani population) as the cases [15,16].

2.2 DNA Isolation and Genotyping

The clinical assessment and DNA isolation were same as described previously [15, 16]. Genotyping of rs7903146 (c.382-41435C>T) was done based on the previously reported primers and methods [17] but with varying amplification conditions. A final volume of 25 µl for the Polymerase Chain Reaction (PCR) contained 2 µl (30-40 ng/µl) of diluted genomic DNA 0.5 mM deoxyribonucleotide triphosphate (dNTPs) (Invitrogen®, Grand Island, NY), 1.25 X ammonium sulphate *Taq* Buffer (Invitrogen®), 3.0 mM $MgCl_2$ (Invitrogen®), *Taq DNA* Polymerase 2.5U/ reaction (Invitrogen®), DNase/RNase free water (Invitrogen®) and 0.24 µM of each allele specific forward and the common reverse primers along with 2.0 µM of the internal control primers. Thermal cycling was done in three steps including initial denaturation at 94°C for 4 min, followed by 32 cycles of amplification at 94°C for 1 min (denaturation), 50°C for 1 min (annealing), 72°C for 1 min (extension), and final extension at 72°C for 5 min and visualized under UV transilluminator using Gel Documentation

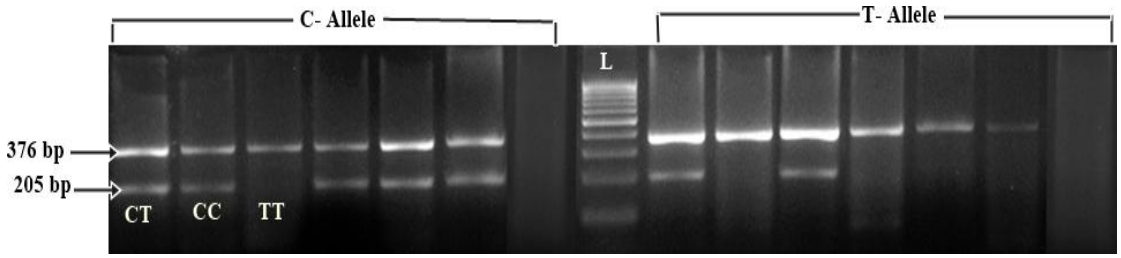

Fig. 1. ARMS-PCR amplification of rs7903146 (c.382-41435C>T). The C-specific and T-specific allele reaction performed on 6 samples with a negative control at the extreme right of both sides of the ladder on 2% gel image. Presence or absence of allele-specific (AS) band at 205 bp confirms genotype, while internal control (IC) at 376 bp is present in all. The size of each fragment is compared with DNA ladder (L) at the center

System (Alpha-Imager Mini Bucher Biotech, Basel, Switzerland). The expected product sizes were allele specific band 205 bp, and 376 bp for the internal control (above Fig. 1).

2.3 Statistical Analysis

The data were statistically analyzed using R software (version 3.2.2; Copyright (C) 2015, The R Foundation for Statistical Computing). The Hardy-Weinberg equilibrium (HWE) was tested using the goodness-of-fit chi-square test using online software (URL: http://www.had2know.com/academics/hardy-weinberg-equilibrium-calculator-2-alleles.html). A P-value of ≤ 0.05 was considered as statistically significant.

3. RESULT AND DISCUSSION

3.1 Result

The SNP in control group was in Hardy Weinberg equilibrium (HWE) 0.935. A significant difference in the genotype frequency distribution among control and T2DM was observed (χ^2=45.84, P=1.11×10^{-10}) where CT (47.20% vs 27.43%) and TT (6.61% vs 2.66%) genotype frequencies are higher in diabetes than in controls and was found to be associated to T2DM under dominant model (DM) with an OR of 3.26 (95% CI 2.24-4.75, P=5.40×10^{-11}); as well as recessive model (RM) OR=4.61 (95% CI 1.88-13.61, P=1.35×10^{-4}). In addition allele frequency allele-T was found to be higher in diabetes (34.78%) cases when compared to healthy controls (χ^2=45.47, P=2.48×10^{-11}; OR=2.72, 95% CI 2.00-3.71, P=6.65×10^{-12}; Table 1).

3.2 Discussion

The *TCF7L2* is reported to harbor intronic variants that are associated with different diseases including T2DM and its complications. In the current case-control study the association of *TCF7L2* genetic polymorphisms (rs7903146) has been assessed in T2DM patients in Pakistani population.

TCF7L2 gene spans 215,869 bp region on the chromosome 10q25.3 and encodes for a transcription factor of the Wnt signaling pathway, it is considered as one of the major genes that plays significant role in β cell development, blood-glucose homeostasis, cell survival, cell migration and cell proliferation [18].

Table 1. Genotype and allele frequency distribution of rs7903146 TCF7L2 (C/T) in T2DM cases and Controls

Genotype	Controls, n = 226	T2DM, n = 322	χ^2 (P Value)	OR (95% CI) P value
CC	158, 69.91%	134, 41.62%		
CT	62, 27.43%	152, 47.20%	45.84(1.11×10^{-10})	DM: 3.26(2.24-4.75) 5.40×10^{-11}
TT	6, 2.66%	36, 6.61%		RM: 4.61(1.88-13.61) 1.35×10^{-4}
Alleles	n = 452	n = 644		
C	378, 83.63%	420, 65.22%	45.47(2.48×10^{-11})	2.72(2.00-3.71) 6.65×10^{-12}
T	74, 16.37%	224, 34.78%		

Legends: χ^2: Chi-square of independence; OR (95%CI): Odds Ratio (95% Confidence Interval); DM: Dominant Model (CT+TT versus CC); RM: Recessive Model (CC+CT versus TT)

The *TCF7L2* gene has seventeen exons, of which five undergo alternative splicing. The highest overall *TCF7L2* gene expression was detected in pancreas, followed by other tissues such as colon, small intestine, brain, monocytes and lungs. Lower expression of *TCF7L2* was observed in T and B lymphocytes [19]. Alternative splicing in *TCF7L2* is predicted to either activate or repress the Wnt signaling pathway [3]. The transcription factor TCF7L2 encoded by *TCF7L2* belongs to TCF family (TCF7, TCF7L1 and TCF7L2) of the Wnt signaling pathway [11]. The interaction of TCF7L2 protein with beta catenin enables the translocation of TCF7L2 from the cytosol to the nucleus; and subsequent binding to promoter region thereby induce gene expression. The TCF7L2 protein mediates the expression of many genes including glucagon like peptide-1 (GLP-1), vascular endothelial growth factor (VEGF) and intercellular adhesion molecule-1 (ICAM-1). The GLP-1 is an incretin (sugar regulating) hormone that mediates insulin secretion [20–22].

It has been observed that the DNA variations in the *TCF7L2* cause over expression of the protein product which results in the GLP-1 dysfunction and hence impair insulin secretion [23]. This malfunction in insulin secretion leads to insulin resistance which is the hallmark of T2DM [20].

T2DM is a complex metabolic disorder of environmental risk factors and genetic susceptibility; it manifests when insulin insufficiency is accompanied by insulin resistance [24]. Recent reports indicated an increase in T2DM cases with its associated complications worldwide, particularly in developing countries. Several studies have investigated the increased risk of T2DM was associated with *TCF7L2* polymorphism and these studies hypothesized the association was due to β cell dysfunction and insulin resistance [8].

A study conducted in Canada revealed the role of *TCF7L2* expression in β cell development and impaired glucose homeostasis in mouse models using functional knockdown approach. The study concluded that *TCF7L2* plays a significant role in pancreatic β cells biogenesis and development [25]. Similarly another study reported the role of *TCF7L2* in *GLP-1* and stromal-derived factor-1 (*SDF-1*) relating the overexpression of these hormones to polymorphisms in *TCF7L2* which lead to insulin resistance [8,26]

The first study on *TCF7L2* polymorphism association with T2DM was conducted in 2006 in Icelandic population, the study reported the comparison of non-risk controls with heterozygous and homozygous cases of the risk alleles for rs12255373 and rs7903146 *TCF7L2* polymorphisms association with risks of T2DM, of which the relative risk of 1.45 and 2.41, respectively were found using Mantel-Haenszel statistical test [9].

Our study supported the association of *TCF7L2* SNPs with T2DM as proposed by Grant et al. [9] (Table 1). Large number of ethnic groups from different cohorts reported the association of *TCF7L2* gene polymorphism with T2DM i.e. Bodhini et al. [27] and Uma Jyothi et al. [7] Independently reported association of *TCF7L2* gene variants with T2DM in Indian population.

Association between *TCF7L2* gene variants and T2DM in UK-resident South Asia, Iran, Lebanon and Japanese cohorts [10,28–31]. Moreover, In Africa; association was reported in Cameroonian population and Ghanaian population [4,32,33]. Our study revealed the association of *TCF7L2* polymorphisms with T2DM in Pakistani population (Table 1). Several researches identified rs7903146 SNP to have strong association [9,34].

In contrast to our study, association of T2DM with *TCF7L2* polymorphisms were not found in some countries like Saudi Arabia, United Arab Emirates and Han Chinese population [14,35, 36].

4. CONCLUSION

In conclusion, our results confirm the association of *TCF7L2* polymorphism with risk of T2DM in Pakistanis; also evidence of association between *TCF7L2* variants confirmed its role as risk factor for the development of diabetes and its complications.

ETHICAL APPROVAL

The case-control associated study was approved by the Ethical Review Board of the Department of Bioscience, COMSATS Institute of Information Technology, in accordance with the Declaration of Helsinki.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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