



## Association of Transcription Factor 7 Like 2 (TCF7L2) rs12255372 (G/T) Gene Polymorphism and Type 2 Diabetes Mellitus

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### Authors' contributions

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

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### ABSTRACT

**Aim:** The rs12255372 of transcription factor 7 like 2 (TCF7L2) was found to be associated with risk of type 2 diabetes mellitus (T2DM) in different populations worldwide. Therefore in the present study we study the relationship between rs12255372 and T2DM in Pakistani population.

**Methodology:** The study comprised of 333 T2DM subjects and 234 normoglycemic control. Genotyping was done using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP).

**Results:** Logistic regression analysis of alltyped data revealed association of rs12255372 with T2DM (odd ratio [OR] =2.30; 95% confidence interval [CI] 1.68-3.16, P=3.14× 10<sup>-8</sup>), also significant association (OR=3.03; 95% CI 2.07-4.43, P=1.00× 10<sup>-9</sup>) was observed in the dominant model (DM). Upon stratified analysis we observed T alleles in females (OR=3.60; 95% CI 1.94-7.13, P=6.70× 10<sup>-7</sup>).

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<sup>6</sup>) to be more associated with the disease compared to males (OR=1.94; 95% CI 1.32-2.87, P=4.56x 10<sup>-4</sup>). GT genotypes of both genders support their allelotypes.

**Conclusion:** The T allele and GT genotype highlight the possible role of rs12255372 in Pakistani T2DM patients.

*Keywords: T2DM; TCF7L2; polymorphism; Wnt.*

## 1. INTRODUCTION

Diabetes is a metabolic disease that is characterized by chronic hyperglycemia due to defect in insulin secretion or action which result into acute and chronic complications [1]. Type 2 diabetes mellitus (T2DM) is the most common form of heterogeneous diabetes which is caused by interactions between genetic and environmental factors [2]. Genome-wide association studies (GWAS) have led to the identification of several susceptible genes to diseases including T2DM [3–5]. Transcription factor 7-like 2 (TCF7L2) is involved in insulin production and secretion via the “wingless” (WNT) signaling pathway in  $\beta$  pancreatic cells. The TCF7L2 gene spans 215,863 bp region on chromosome 10q25.3 [6]. GWAS reported a relationship between a common micro-satellite region (DG10S478) in intron 3 of the TCF7L2 gene and T2DM [7,8]. In addition, several studies identified other polymorphisms of TCF7L2 gene associated to T2DM, amongst which are rs7903146 (C/T), rs7901695 (T/C), and rs12255372 (G/T) [1]. In the present study we assess the association of rs12255372 TCF7L2 with T2DM in Pakistani population.

## 2. MATERIALS AND METHODS

### 2.1 Sampling

The whole blood samples of cases were collected from collaborating hospitals in Islamabad and Rawalpindi cities of Pakistan. In the current case-control association analysis all cases were clinically diagnosed for T2DM by a professional endocrinologist and had T2DM for over 10 years. Patients were diagnosed based on the American Diabetic Association criteria for the diagnosis of T2DM i.e. age 18–75years, fasting plasma glucose level  $\geq$  126 mg/dl, random plasma glucose concentration  $\geq$  200 mg/dl, and serum creatinine concentration  $\leq$  2.0 mg/dl. The controls were sampled from the same general population as the cases.

### 2.2 Genotyping

Genomic DNA was extracted from 2 ml whole blood using phenol chloroform method and genotyped using PCR followed by Restriction Fragment Length Polymorphism (RFLP)[1]. Amplification reactions were performed in a total volume of 25  $\mu$ l which contained 2  $\mu$ l (40-50 ng/ $\mu$ l) of the genomic DNA, 0.25 mM dNTPs (Invitrogen<sup>®</sup>, Grand Island, NY), 1.25 X ammonium sulphate ((NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>) Taq Buffer (Invitrogen<sup>®</sup>), 2.0 mM MgCl<sub>2</sub> (Invitrogen<sup>®</sup>), Taq DNA Polymerase 2.5U/ reaction (Invitrogen<sup>®</sup>), DNase/RNase free water (Invitrogen<sup>®</sup>) and 0.3  $\mu$ M of forward and reverse primer each. The thermal cycling profile was as follows: initial denaturation at 95°C for 2min, followed by 35 cycles of 95°C for 30 sec (denaturation), 56°C for 30 sec (annealing), 72°C for 30 sec (extension), and a final extension of 72°C for 5 min using thermocycler (Thermo Electron Corporation). Amplified PCR products (346 bp) were then digested with the *Tsp509I* restriction enzyme based on manufacturer’s protocol (Invitrogen<sup>®</sup>, Grand Island, NY). Briefly, the reaction volume of 15  $\mu$ l contained 7  $\mu$ l of amplicons, 1x reaction buffer B, 1Unit of *Tsp509I*, and 6.3  $\mu$ l of nuclease free water. The digested products were then separated by electrophoresis on an ethidium bromide (10 mg/mL) containing 3.5% agarose gel and visualized under UV transilluminator using Gel Documentation System (Alpha-Imager Mini Bucher Biotech, Basel, Switzerland). The expected product sizes after digestion were wild type GG 143bp, 104bp; mutant homozygous TT 126bp, 104bp and heterozygous GT 143bp, 126bp, 104bp respectively. The digested product fragments smaller than 100 bp were not visible.

### 2.3 Statistical Analysis

Statistical calculations were performed 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 with online software (URL:

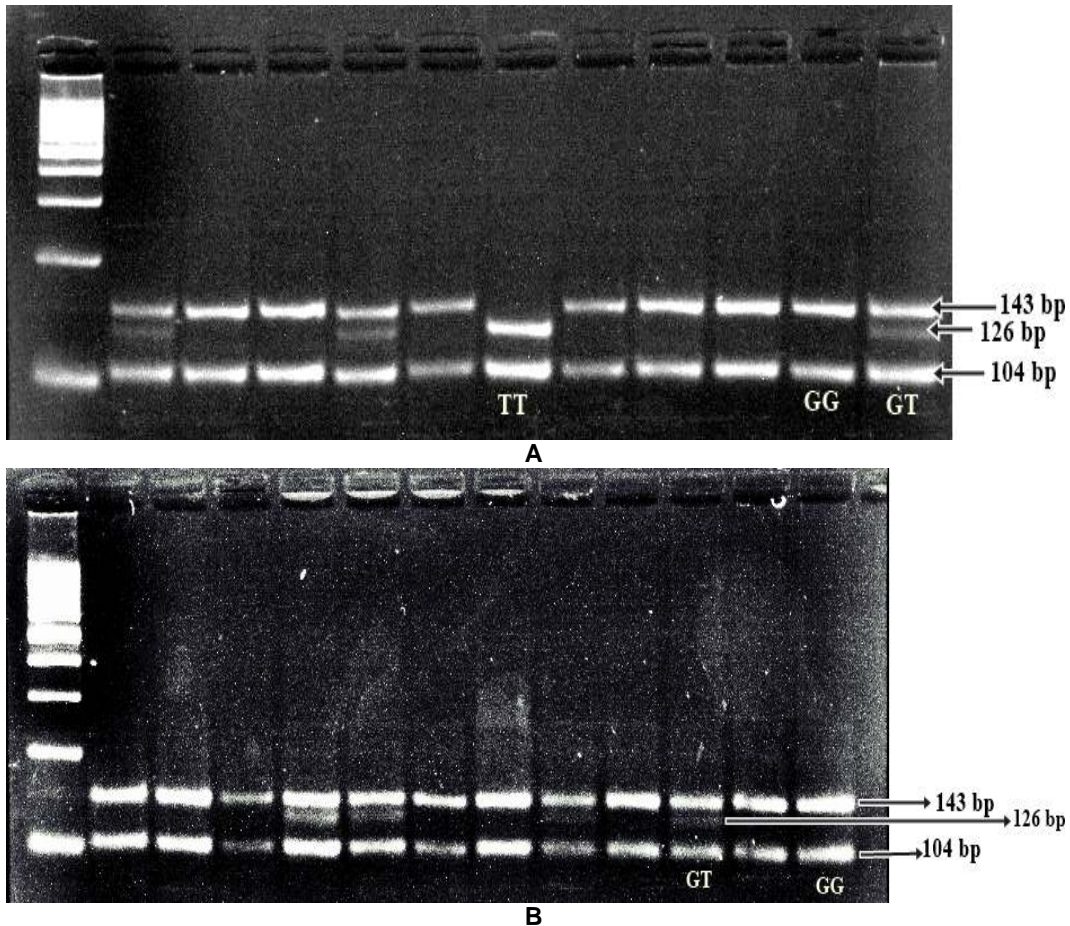
<http://www.had2know.com/academics/hardy-weinberg-equilibrium-calculator-2-alleles.html>). A P-value of  $\leq 0.05$  was considered as statistically significant.

### 3. RESULTS AND DISCUSSION

#### 3.1 Results

The rs12255372 was genotyped in 567 individuals involving 333 cases and 234 controls in Pakistani population. Significant deviation was observed from Hardy Weinberg equilibrium (HWE) in control group ( $p < 0.05$ ; Table 1). A significant difference was found in GG ( $Z = 6.07$ ,  $P = 1.31 \times 10^{-9}$ ) and GT ( $Z = -5.67$ ,  $P = 1.39 \times 10^{-9}$ ) in T2DM when compared to controls. The genotype

distribution of rs12255372 revealed significant difference ( $\chi^2 = 37.21$ ,  $P = 8.31 \times 10^{-9}$ ) between cases and control and GT was found to be associated with T2DM under dominant model (DM) with an OR of 3.03 (95% CI 2.07-4.43;  $P = 1.00 \times 10^{-9}$ ). A statistical association was also observed in the G and T alleles in T2DM cases and control groups ( $\chi^2 = 29.77$ ,  $P = 7.26 \times 10^{-8}$ ; OR = 2.30, 95% CI 1.68-3.16,  $P = 3.14 \times 10^{-8}$ ; Table 1). Upon stratified analysis, females (OR = 3.60; 95% CI 1.94-7.13,  $P = 6.70 \times 10^{-6}$ ; Table 3) were found to be more associated with T2DM than males (OR = 1.94; 95% CI 1.32-2.87,  $P = 4.56 \times 10^{-4}$ ; Table 2). A significant difference ( $P \leq 0.05$ ) was observed in between the GG and GT of cases and control groups in both male and female (Table 2 and 3).



**Fig. 1.** The PCR-RFLP detection of *TCF7L2* rs12255372 (c.482+901G>T) polymorphism PCR followed by digestion with *Tsp509I*- 3.5% agarose gel electrophoresis followed by ethidium staining and UV trans-illuminator performed shown in (A) T2DM and (B) control groups. The expected product sizes after digestion were: normal homozygote GG, 143 bp, 104 bp; mutant homozygote TT, 126 bp, 104 bp; and heterozygote GT, 143, 126, and 104 bp, respectively. The digested product fragments smaller than 100 bp were not visualized

**Table 1. Genotype and allele frequency distribution of rs12255372 TCF7L2 (G/T) in T2DM cases and Controls**

Genotype	Controls, n = 234	T2DM, n = 333	$\chi^2$ (P Value)	Z test (P Value)	OR (95% CI) P Value
GG	174(74.36%)	163(48.95%)		<b>6.07(1.31× 10<sup>-9</sup>)</b>	
GT	50(21.37%)	148(44.44%)	<b>37.21(8.31× 10<sup>-9</sup>)</b>	<b>-5.67(1.39× 10<sup>-9</sup>)</b>	<b>DM:3.03(2.07-4.43) 1.00× 10<sup>-9</sup></b>
TT	10(4.27%)	22(6.61%)		-1.19(23.40 × 10 <sup>-2</sup> )	RM:1.59(0.70-3.66) 27.10× 10 <sup>-2</sup>
Alleles	n = 468	n = 666			
G	398(85.04%)	474(71.17%)	<b>29.77(7.26× 10<sup>-8</sup>)</b>		<b>2.30(1.68-3.16) 3.14× 10<sup>-8</sup></b>
T	70(14.96%)	192(28.83%)			

Legends:  $\chi^2$ : Chi-square of independence; OR (95%CI): Odds Ratio (95% Confidence Interval); DM: Dominant Model (GT+TT versus GG); RM: Recessive Model (GG+GT versus TT)

**Table 2. Genotype and allele frequency distribution of rs12255372 TCF7L2 (G/T) in T2DM cases and Controls for Males**

Genotype	Controls, n = 167	T2DM, n = 171	$\chi^2$ (P Value)	Z test (P Value)	OR (95% CI) P Value
GG	118(70.66%)	87(50.88%)		<b>-3.72(2.00× 10<sup>-4</sup>)</b>	
GT	42(25.15%)	72(42.10%)	<b>13.85(9.82× 10<sup>-4</sup>)</b>	<b>3.30(9.6× 10<sup>-4</sup>)</b>	<b>DM:2.32(1.45-3.74) 2.32× 10<sup>-4</sup></b>
TT	7(4.19%)	12(7.02%)		1.13(0.26)	RM:1.72(0.61-5.30) 0.35
Alleles	n = 334	n = 342			
G	278(83.23%)	246(71.93%)	<b>11.75(6.09× 10<sup>-4</sup>)</b>		<b>1.94(1.32-2.87) 4.56× 10<sup>-4</sup></b>
T	56(16.77%)	96(28.07%)			

Legends:  $\chi^2$ : Chi-square of independence; OR (95%CI): Odds Ratio (95% Confidence Interval); DM: Dominant Model (GT+TT versus GG); RM: Recessive Model (GG+GT versus TT)

**Table 3. Genotype and allele frequency distribution of rs12255372 TCF7L2 (G/T) in T2DM cases and Controls for Females**

Genotype	Controls, n = 67	T2DM, n = 162	$\chi^2$ (P Value)	Z test (P Value)	OR (95% CI) P Value
GG	56(83.58%)	76(46.91%)		<b>-5.11(3.24× 10<sup>-7</sup>)</b>	
GT	8(11.94%)	76(46.91%)	<b>27.10(1.30× 10<sup>-6</sup>)</b>	<b>4.99 (5.85× 10<sup>-7</sup>)</b>	<b>DM:5.72(2.72-13.01) 1.78× 10<sup>-7</sup></b>
TT	3(4.48%)	10(6.18%)		0.50(0.62)	RM:1.40(0.35-8.18) 0.76
Alleles	n = 134	n = 324			
G	120(89.55%)	228(70.37%)	<b>18.08(2.12× 10<sup>-5</sup>)</b>		<b>3.60(1.94-7.13) 6.70× 10<sup>-6</sup></b>
T	14(10.45%)	96(29.63%)			

Legends:  $\chi^2$ : Chi-square of independence; OR (95%CI): Odds Ratio (95% Confidence Interval); DM: Dominant Model (GT+TT versus GG); RM: Recessive Model (GG+GT versus TT)

### 3.2 Discussion

The study examined the association between rs12255372 TCF7L2 gene polymorphism and T2DM in Pakistani population. Though the disease progression is due to interplay of environmental factors and genetic predisposition, TCF7L2 gene has been considered most significant genetic determinant for the risk of developing T2DM and its microvascular complications such as diabetic retinopathy [9], neuropathy [10] and nephropathy [11]; other diseases associated to TCF7L2 are cardiovascular disease [12,13], cancer [14,15] and wound healing [8]. TCF7L2 gene encodes a transcription factor in Wnt signaling pathway, expressed in several tissues including pancreas, known to have developmental roles in determining cell fate, survival, proliferation and movement [16–18]. Wnt signaling plays an important role in B-cell proliferation and insulin secretion by influencing synthesis of glucagon-like peptide 1 (GLP-1) [19,20]. The current study confirmed the role of TCF7L2 gene in the development of T2DM in Pakistani population.

GWAS on *TCF7L2* polymorphisms association with T2DM 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 [7,21].

A study using functional knockdown approach revealed the role of *TCF7L2* expression in  $\beta$  cell development and impaired glucose homeostasis in mouse models which concluded that *TCF7L2* plays a significant role in pancreatic  $\beta$  cells biogenesis and development [22]. 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 [23,24].

Our result is successful replication of the previous finding of association between rs12255372 and T2DM [1,25–30]. However, there are some investigations which reported weak or no association between rs12255372 and T2DM [31,32]. The Hardy-Weinberg equilibrium (HWE) was in violation with the general population. Deviation from HWE in this study was confirmed by replicated genotyping using fresh reagents which yield the same outcome. This was also reported in previous studies [1,28]. The high odd ratio and level of statistical significance

found in heterozygous GT and risk T allele are clear indication that there is probably an association between rs12255372 TCF7L2 and T2DM in Pakistani population.

### 4. CONCLUSION

The rs12255372 (G/T) polymorphism of the TCF7L2 gene is probably associated with T2DM in this population. This variant could help to predict the occurrence of T2DM in the Pakistani population. Our findings should be confirmed with more accurate genotyping tools.

### CONSENT AND ETHICAL CONSIDERATION

The current case-control association study was approved by Ethics Review Board of the Department of Biosciences; COMSATS Institute of Information Technology (CIIT), Islamabad Pakistan. Prior to blood sampling, the objectives of the study were clearly explained to the participants and written consents were taken from them.

### COMPETING INTERESTS

Authors have declared that no competing interests exist.

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