

Lack of Genetic Association between SNP Rs7903146 (IVS3C>T) of Transcription Factor 7 Like 2 (TCF7L2) Gene in Type 2 Diabetes Mellitus in Pakistani Population

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ABSTRACT

Background: Despite the high prevalence of the type 2 diabetes in South Asians, very few studies carried out for genetic predisposition of disease susceptibility in Pakistani population.

Aim: To determine the association of polymorphism rs7903146 (IVS3C>T) of TCF7L2 gene among T2DM patients and controls.

Methods: A total of 170 diabetic patients and 170 healthy controls were included in the study. The whole blood was collected from each participant and genomic DNA extraction was carried out by standard phenol-chloroform method. PCR amplification of unique oligonucleotides was performed and restriction fragment length polymorphism (RFLP) was done by using restriction enzyme *SspI*. Data was analyzed by SPSS and p-value <0.05 was considered statistically significant. **Results:** The results showed that there was no association for genotype and allele distribution between T2DM cases and controls for rs7903146 (IVS3C>T) polymorphism 0.67 (95% CI, 0.42-1.05 OR, p= 0.08) compared with the CC genotype, respectively.

Conclusion: The results showed that no genetic association of TCF7L2 established with the disease susceptibility of type 2 diabetes in Pakistani population.

Keywords: Type 2 Diabetes Mellitus, genetic polymorphism, TCF7L2, Pakistan

INTRODUCTION

Diabetes is a multifactorial disease, presents in every home and is increasing very rapidly all over the world. It causes serious complications that may lead to death. Genes and environment interrelation are making it a complex and polygenic disease. Susceptible gene recognition is mandatory for the prevention of ailment. More than 120 variants have been narrated that are in association with diabetes¹. TCF7L2 is the most widely studied gene. Many mechanisms have been proposed but still, there is no specific mechanism which fully explains the route of the pathogenesis of susceptible gene². The current notion about its pathogenicity is via impaired insulin secretion, enteroinsular axis and increased gene expression in islets^{3,4}.

Transcription Factor 7-Like 2 Gene (TCF7L2) was previously known as Transcription Factor 4 (TCF-4) and belongs to transcription factors (TCF) family. It is a protein-coding gene and produces TCF7L2 protein, which is a transcription factor involved in the coding of multiple genes. The gene is highly expressed in cells of many organs like liver, adipose tissues, and pancreatic cells⁵, where many developmental processes take place⁶. It is proposed that TCF7L2 confer diabetes by beta cell dysfunctioning^{4,7}.

Higher frequency of T allele is associated with increased expression of TCF7L2 in pancreatic β-cells. The protein content of the TCF7L2 gene increases up to 5 folds in diabetic islets, especially in TT homozygotic carriers.

Over expression of respective gene leads to increased insulin gene expressions in β-cells and shows positive correlation but also a negative correlation seems between TCF7L2 protein expression and stimulation index i.e glucose-stimulated insulin secretion. The increase in insulin gene expression may be a result of a compensatory mechanism due to a decrease in insulin release. Risk T allele for SNP 7903146 is also associated with raised endogenous glucose production than to non-risk CC genotype. This may be due to the impaired secretion of insulin in T allele carriers⁴. The animal model study revealed that if TCF7L2 is overexpressed in the liver, it causes hyperglycemia and in case of displacement of the gene in liver cells results in hypoglycemia⁸.

Although, the high prevalence of the disease in South Asians, the data for risk involvement of rs7903146 SNP of TCF7L2 in type 2 diabetic patients is rare in Pakistani population. The objective of this study was to determine the association of single nucleotide polymorphism of rs7903146 of TCF7L2 gene in T2DM patients of teaching hospital of Lahore.

METHODS

A total of 340 subjects were recruited including 170 T2DM patients and 170 healthy controls. The age of cases was between 35 to 55 years and controls were 25 to 55 years. Patients with type 1 DM were excluded from the study. Ethical permission was obtained from institution and written consent was taken from each participant for sample collection. A 5 ml of whole blood was collected for genetic and biochemical analysis. The levels of fasting blood

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glucose were determined in T2DM patients and healthy controls.

Genotyping of *TCF7L2* in T2DM patients and controls:

Total genomic DNA was extracted by standard phenol-chloroform method. Amplification of unique primers for SNP rs7903146 (F-5'-ACAATTAGAGAGCTAAGCACTTTTAAATA-3'; R-5'-CTAACCTTTCTAGTTATGACATTG-3') was carried out by PCR. PCR tube for 25 µl reaction volume contained 8 µl of Master mix (Dream Tag green 2X), forward and reverse primers of each 0.5 µl (10pmol/µl), 1 µl of template DNA and nuclease-free water to 15 µl reaction volume. PCR conditions on Thermo-cycler were set as the following: digestion at 95°C for 5 minutes; 35 cycles of digestion at 95°C for 30 seconds, annealing at 59°C for 30 seconds, extension at 72°C for 30 seconds and a final extension for 10 minutes at 72°C, and then stored at -4°C for further processing.

For PCR product sizing, a ladder of 50 bp was run along with the amplified products on 2% agarose gel. Restriction fragment length polymorphism was carried out by using *SspI*, restriction enzyme (1 µl of enzyme, 2 µl of 10 X buffer, 4µl of PCR product, 8 µl of nuclease-free water and incubation at 37°C for overnight). The digested products were resolved on 3% agarose with 50 bp ladder and bands were visualized by Gel documentation system (BioRad). Interpretation of fragments was done as heterozygous or homozygous genotype.

Statistical analysis: Data analysis was performed by Statistical Package for Social Sciences version 20.0 and descriptive data was presented in frequency and percentages. Quantitative variables like age and glucose were given as mean ± standard deviation. A qualitative variable such as gender distribution and polymorphism rs 7903146 was displayed in frequencies and percentages. Genotype and allele frequency was determined in both groups by IBM SPSS Software (Version 20.0, SPSS Inc) and their Odds were calculated with 95% CI. Hardy-Weinberg equilibrium was calculated by using OEGE-Online Encyclopedia for Genetic Epidemiology Study. Genetic modeling and their respective ODDS with a 95% confidence interval (CI) was done by using Snpstats online

software. P-value < 0.05 was considered statistically significant for odd ratio.

RESULTS

This study recruited 170 confirmed T2DM patients from the Diabetic Clinic of Sheikh Zayed Hospital, Lahore and 170 non-diabetic volunteers subjects from University of Health Sciences, Lahore, were included as non-related controls, without the family history of diabetes to evaluate the *TCF7L2* gene rs 7903146 polymorphism. Both groups were of natively Pakistani Ancestry. Among 170 cases, 65 were males (38.2%) and 105 (61.8%) were females. Among 170 controls, 110 (64.7%) were male and 60 (35.2%) were females. Cases with the mean age±standard deviation of 50.07±10.16 years. Controls with mean age ± standard deviation of 32.62±8.2. 121/200 (61%) cases were having a family history of diabetes. Age and gender differed between both groups. Mean of BSF was 199.1±73.11 and BSR 233.43±82.01 mg/dL, statistically not a significant difference.

Restriction was done and the distribution of bands was visualized and interpretation was done which is presented in Figure 1. The genotype frequency distributions of the *TCF7L2* rs7903146 (IVS3C>T) polymorphism were 55.9 % (CC), 32.3 % (CT), and 11.8 % (TT) in the cases and 50.0% (CC), 43.5% (CT), and 6.5% (TT) in the controls as presented in table 1. Also, there was no association found between allele frequencies in cases and controls (OR= 0.9855, 95%CI, OR= 0.7054-1.3770, p 0.93). The frequency of allele T was 27.9% in cases and 28.2% in controls, while the frequency of C allele was 72.1% in cases and 71.8% in controls (Table 2). After adjustment for age, gender and biochemical profile, the association between the *TCF7L2* rs7903146 (IVS3C>T) polymorphism and T2DM patients was analyzed. In a co-dominant model, the homozygous CT genotype of *TCF7L2* rs7903146 (IVS3C>T) polymorphism was contributed to 32.4% occurrence of T2DM (OR=0.62, 95% CI=0.40-0.97, p 0.28). In dominant model, the heterozygous CT/TT genotypes of rs7903146 polymorphism contributed to 44.1% occurrence of T2DM (OR=0.79, 95% CI= 0.52-1.21, p<0.001) and after adjustment (OR=1.39, 95% CI=0.81-7.04, p 0.53) presented in Table 3.

Table 1: Genotype frequencies of single nucleotide polymorphisms in *TCF7L2* gene in T2DM patients and control subjects of Lahore region in Pakistan.

rs7903146 (IVS3C>T)	T2DM Patients (n=170)	Controls (n=170)	p value	OR Unadjusted (95% CI)	p value	OR Adjusted (95% CI)
CC	95 (55.9%)	85 (50%)	0.08	1.00	0.14	1.00
CT	55 (32.4%)	74 (43.5%)		0.67 (0.42-1.05)		2.00 (0.65-6.16)
TT	20 (11.8%)	11 (6.5%)		0.63 (0.74-3.59)		0.35 (0.06-2.10)

p values were calculated by chi-square test (Test for Independence), p<0.05 significant

Table 2: Allele frequencies of single nucleotide polymorphisms (rs7903146) in *TCF7L2* gene in T2DM patients and control subjects of Lahore region in Pakistan

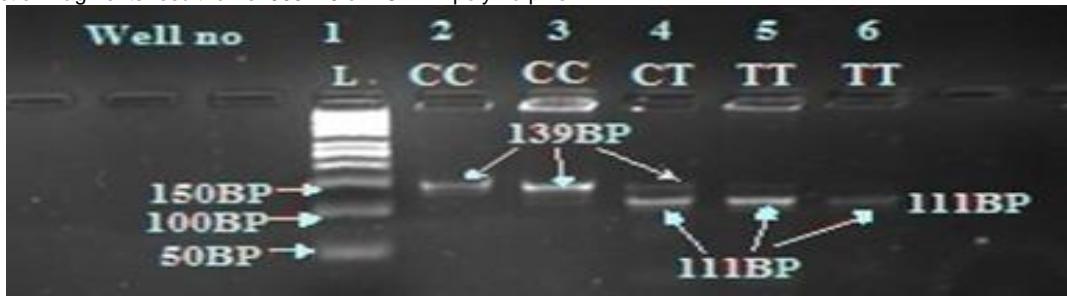
rs7903146 (IVS3C>T)	Patients (n=170)	Controls (n=170)	*p value = 0.93 OR= 0.9855 95%CI= 0.7054-1.3770
T allele	95 (27.9%)	96 (28.2%)	
C allele	245 (72.1%)	246 (71.8%)	

OR, Odds ratio for A allele frequency in all subjects; 95%CI, confidence interval

Table 3: Genotype models for disease association of *TCF7L2* gene (rs7903146) in T2DM patients and control subjects of Lahore region in Pakistan

rs7903146 (IVS3C>T)	Patients (n=170)	Controls (n=170)	p value	OR Unadjusted (95% CI)	p value Adjusted	OR Adjusted (95% CI)
Dominant model CC	95 (55.9%)	85 (50%)	0.28	0.79 (0.52-1.21)	0.53	1.39 (0.49-3.91),
CT+ TT	75 (44.1%)	85 (50%)				
Recessive Model CC+ CT	150 (88.2%)	159 (93.5%)	0.08	1.93 (0.89-4.16)	0.12	0.26 (0.05-1.45)
TT	20 (11.8%)	11 (6.5%)				
Co-dominant model CC+TT	115 (67.7%)	96 (56.6%)	0.0348*	0.62 (0.40-0.97)	0.11	2.39 (0.81-7.04)
CT	55 (32.4%)	74 (43.5%)				

*p<0.05 significant

Fig. 1: Restriction fragments result for rs7903146 of *TCF7L2* polymorphism.

The well no 1 contain 50 bp ladder (L). Well no 2 and 3 show one band of 139 bp, so both are CC homozygous. Well no 4 depicts 2 bands, one of 139 bp and lower one of 111 bp so it is heterozygous CT. Well no 5 and 6 show only one band of 111 bp, so representing TT homozygous genotype.

DISCUSSION

The association between rs7903146 of *TCF7L2* gene and T2DM has been investigated worldwide and considered as an important genetic risk factor for the disease development. Several studies demonstrated the association of *TCF7L2* rs7903146 (IVS3C>T) polymorphism and T2DM⁹⁻¹⁸. In a meta-analysis study, association of *TCF7L2* polymorphism rs7903146 was linked to T2DM susceptibility by comparing different genetic model analysis, they recommended that to include well designed larger sample size studies to increase the power of their conclusion¹⁹.

In present study, we investigated 170 cases of T2DM for genetic association for rs7903146 of *TCF7L2* gene. The genotype and allele distribution of *TCF7L2* gene was not associated with disease susceptibility of T2DM in our population. Although, these results are in consistent with previous studies, further exploration the exact role of this gene is required. In Chinese population, different studies demonstrated no genetic association of rs7903146 of *TCF7L2* to the risk of T2DM^{20, 21}. In another study from Han Chinese population, the association of single study was failed to establish between SNP and T2DM but the analysis was combined with the different studies of Chinese origin and then the association was established to the risk of T2DM²².

In contrast to our study, previous reports from Punjabi population documented the association of *TCF7L2* with T2DM^{13, 23, 24}. Although, association has been demonstrated in some reports from Punjabi population, but the reported population is mixed UK-Punjabi (Azad Jammu & Kashmir) population. Therefore, it may be explained that the Punjabi

population of Pakistan is diverse and consist of various language groups. The present study conducted on the Punjabi population residing in the Lahore from various decades and is not mixed ancestry. Hence the difference in the association is might be due to the variation in environmental, genetic factors, by ethnicity differences.

CONCLUSION

It is concluded that no association between rs7903146 of *TCF7L2* gene and T2DM in Punjabi (Lahore City) population of Pakistan. Although T allele is prevalent in our population but significant association cannot be established. This showed that there is disease heterogeneity in multiple populations. These types of studies are required on larger scale to establish the exact mechanism of disease pathogenicity and may be helpful for management purposes.

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REFERENCES

- Prasad, R. B., Groop, L. Genetics of type 2 diabetes-pitfalls and possibilities. *Genes (Basel)*, 2015; **6**(1): 87-123. doi: 10.3390/genes6010087.
- Pourahmadi, M., Erfanian, S., Moradzadeh, et al. Non-Association between rs7903146 and rs12255372

- Polymorphisms in Transcription Factor 7-Like 2 Gene and Type 2 Diabetes Mellitus in Jahrom City, Iran. *Diabetes Metab. J.*, 201; **39**(6): 512-517. doi: 10.4093/dmj.2015.39.6.512.
3. Villareal, D. T., Robertson, H., Bell, G. I., et al. *TCF7L2* variant rs7903146 affects the risk of type 2 diabetes by modulating incretin action. *Diabetes*, 2010; **59**(2): 479-85. doi: 10.2337/db09-1169.
 4. Lysenko, V., Lupi, R., Marchetti, P., et al. Mechanisms by which common variants in the *TCF7L2* gene increase risk of type 2 diabetes. *J. Clin. Invest.*, 2007; **117**(8): 2155-63.
 5. Zhou, Y., Park, S. Y., Su, J., et al. *TCF7L2* is a master regulator of insulin production and processing. *Hum. Mol. Genet.*, 2014; **23**(24): 6419-31. doi: 10.1093/hmg/ddu359.
 6. Jin, T., Liu, L. The Wnt signaling pathway effector *TCF7L2* and type 2 diabetes mellitus. *Mol. Endocrinol.*, 2008; **22**(11): 2383-92. doi: 10.1210/me.2008-0135.
 7. Sun, X., Yu, W., Hu, C. Genetics of type 2 diabetes: insights into the pathogenesis and its clinical application. *Biomed. Res. Int.*, 2014; **2014**: 926713. doi: 10.1155/2014/926713.
 8. Boj, S. F., Van Es, J.H., Huch, et al. Diabetes risk gene and Wnt effector *TCF7L2/TCF4* controls hepatic response to perinatal and adult metabolic demand. *Cell*, 2012; **151**(7): 1595-1607. doi: 10.1016/j.cell.2012.10.053.
 9. Scott, L. J., Bonnycastle, L. L., Willer, C. J., et al. Association of transcription factor 7-like 2 (*TCF7L2*) variants with type 2 diabetes in a Finnish sample. *Diabetes*, 2006; **55**(9): 2649-2653.
 10. Groves, C. J., Zeggini, E., Minton, J., et al. Association analysis of 6,736 U.K. subjects provides replication and confirms *TCF7L2* as a type 2 diabetes susceptibility gene with a substantial effect on individual risk. *Diabetes*, 2006; **55**(9): 2640-2644.
 11. Damcott, C.M., Pollin, T. I., Reinhart, L. J., et al. Polymorphisms in the transcription factor 7-like 2 (*TCF7L2*) gene are associated with type 2 diabetes in the Amish: replication and evidence for a role in both insulin secretion and insulin resistance. *Diabetes*, 2006; **55**(9): 2654-2659.
 12. Hayashi, T., Iwamoto, Y., Kaku, K., et al. Replication study for the association of *TCF7L2* with susceptibility to type 2 diabetes in a Japanese population. *Diabetologia*, 2007; **50**(5): 980-984.
 13. Rees, S. D., Bellary, S., Britten, A. C., et al. Common variants of the *TCF7L2* gene are associated with increased risk of type 2 diabetes mellitus in a UK-resident South Asian population. *BMC Med. Genet.*, 2008; **9**: 8. doi: 10.1186/1471-2350-9-8.
 14. Buraczynska, M., Swatowski, A., Markowska-Gosik, D., et al. Transcription factor 7-like 2 (*TCF7L2*) gene polymorphism and complication/comorbidity profile in type 2 diabetes patients. *Diabetes Res. Clin. Pract.*, 2011; **93**(3): 390-395. doi: 10.1016/j.diabres.2011.05.017.
 15. Palizban, A., Nikpour, M., Salehi, R., et al. Association of a common variant in *TCF7L2* gene with type 2 diabetes mellitus in a Persian population. *Clin. Exp. Med.*, 2012; **12**(2): 115-9. doi: 10.1007/s10238-011-0144-7.
 16. Uma Jyothi, K., Jayaraj, M., Subburaj, K. S., et al. Association of *TCF7L2* gene polymorphisms with T2DM in the population of Hyderabad, India. *PLoS One*, 2013; **8**(4): e60212. doi: 10.1371/journal.pone.0060212.
 17. Angheben-Oliveira, M. I., Martins, B.R., Alberton, D. Type 2 diabetes-associated genetic variants of FTO, LEPR, PPAR γ , and *TCF7L2* in gestational diabetes in a Brazilian population. *Arch Endocrinol Metab*. 2017; **61**(3):238-248.
 18. <https://doi.org/10.1590/2359-3997000000258>
 19. Cai, J., Zhang, Y., Nuli, R., et al. Interaction between dietary patterns and *TCF7L2* polymorphisms on type 2 diabetes mellitus among Uyghur adults in Xinjiang Province, China. *Diabetes Metab Syndr Obes*. 2019; **12**: 239-255. doi: 10.2147/DMSO.S191759.
 20. Guan, Y., Yan, L. H., Liu, X. Y., et al. Correlation of the *TCF7L2* (rs7903146) polymorphism with an enhanced risk of type 2 diabetes mellitus: a meta-analysis. *Genet Mol Res*, 2016; **15**(3). doi: 10.4238/gmr.15037969.
 21. Zheng, X., Ren, W., Zhang, S., et al. Association of type 2 diabetes susceptibility genes (*TCF7L2*, *SLC30A8*, *PCSK1*, and *PCSK2*) and proinsulin conversion in a Chinese population. *Mol. Biol. Rep.*, 2012; **39**(1): 17-23. doi: 10.1007/s11033-011-0705-6.
 22. Chang, Y. C., Chang, T. J., Jiang, Y. D., et al. Association study of the genetic polymorphisms of the transcription factor 7-like 2 (*TCF7L2*) gene and type 2 diabetes in the Chinese population. *Diabetes*, 2007; **56**(10): 2631-2637.
 23. Wang, J., Hu, F., Feng, T., et al. Meta-analysis of associations between *TCF7L2* polymorphisms and risk of type 2 mellitus in the Chinese population. *BMC. Med. Genet.* 2013; **12**:14:8. doi: 10.1186/1471-2350-14-8.
 24. Zia, A., Wang, X., Bhatti, A. I., et al. A replication study of 49 Type 2 diabetes risk variants in a Punjabi Pakistani population. *Diabet Med.* 2016; **33**(8): 1112-1117. doi: 10.1111/dme.13012.
 25. Rees, S. D., Hydrie, M. Z., Shera, A. S., et al. Replication of 13 genome-wide association (GWA)-validated risk variants for type 2 diabetes in Pakistani populations. *Diabetologia*, 2011; **54**(6): 1368-1374. doi: 10.1007/s00125-011-2063-2.