

## Association of variants of the TCF7L2 gene at rs7903146(C/T) and rs12255372(G/T) with type 2 diabetes mellitus in Chennai suburban population

B. Lavanya Devi<sup>1</sup>, V. Meera<sup>2</sup>, R. Nagendran<sup>3</sup>, R. Lalitha<sup>4</sup>, G. Komala<sup>5\*</sup>

<sup>1</sup>Assistant Professor, <sup>2-4</sup>Professor, <sup>5</sup>Associate Professor, Dept. of Biochemistry, <sup>1</sup>Madras Medical College, Chennai, Tamil Nadu, <sup>2-4</sup>Kilpauk Medical College, Chennai, Tamil Nadu, <sup>3</sup>Moogambigai Medical College, Tamil Nadu, <sup>5</sup>Madras Medical College, Tamil Nadu, India

**\*Corresponding Author: G. Komala**

Email: drgkomala@gmail.com

Received: 17<sup>th</sup> February, 2019

Accepted: 16<sup>th</sup> March, 2019

### Abstract

**Background:** An effective  $\beta$ -catenin/Transcription Factor 7 Like 2 (TCF7L2) axis is essential in transcription of Proglucagon gene for synthesis of GLP-1. It upholds  $\beta$ -cell health, augments glucose-dependent insulin processing, secretion and also suppression of postprandial glucagon secretion. TCF7L2 gene polymorphism at rs7903146 and rs12255372 which are in strong linkage disequilibrium with microsatellite DG 10S478 had shown robust association with T2DM in various population. Hence we aimed to study association between TCF7L2 gene polymorphism and T2DM among Chennai suburban population.

**Materials and Methods:** This case-control study included 44 T2DM cases with 44 age and gender matched healthy controls. Genotyping done by Restriction Fragment Length Polymorphism- Polymerase Chain Reaction for rs7903146(C/T) and by Allele specific Amplification Refractory Mutation System for rs12255372(G/T). By Hardy-Weinberg law, frequency of genotype distribution were compared using Chi-square ( $\chi^2$ ) test.

**Result:** At rs7903146 (C/T), T allele frequency was significantly higher in diabetic group (30.7%) than control group (2.0%) with odds ratio of 0.19 (95% CI 0.04-0.83,  $p < 0.001$ ). Whereas at rs12255372, T allele frequency in cases and control were 23.9% and 15.9%, with odds ratio 1.66 (95% CI 0.78-3.53,  $p = 0.093$ ) showing no significant difference. Genotype distribution obeyed Hardy-Weinberg law being in equilibrium. This study found significant association of TCF7L2 gene polymorphism at rs7903146 with T2DM which affect GLP-1 gene expression leading to increased risk of diabetes. TCF7L2 gene polymorphism at rs12255372 (G/T) did not show association with T2DM in this population.

**Conclusion:** Our finding support that as in many ethnic groups, the TCF7L2 gene polymorphism at rs7903146 (C/T) could be an important genetic risk factor for T2DM in Chennai suburban population.

**Keywords:** Type 2 diabetes, Transcription factor 7-like 2 (TCF7L2), Glucagon like peptide-1 (GLP-1), Insulin, Restriction Fragment Length Polymorphism (RFLP), Allele specific Amplification Refractory Mutation System (ARMS- PCR).

### Introduction

Diabetes Mellitus is an emerging modern epidemic which is threatening health system of many nation. According to International Diabetic Federation (IDF), the prevalence of Diabetes was about 415 million in 2015 which may expand to 642 million in 2040.<sup>1</sup> Also in 2015, Diabetes prevalence in urban environment was 269.7 million which is high compared to rural environment with 145.1 million. Hence, Type 2 Diabetes Mellitus (T2DM) stands as the most challenging health problem in this 21<sup>st</sup> century. In current situation many research is needed about diabetes because of its irreversible consequences such as reduced life expectancy by nearly 15 years, increased risk of cardiovascular disease by 2 to 4 times, and as the leading cause for kidney failure, lower limb amputations and adult – onset blindness. Only by early detection and modification of genetic and environmental factors, one can either delay or even prevent the onset of T2DM. India being one of the countries with largest diabetic population among many ethnic groups, a detailed study on various genetic factors of T2D on every ethnic group is thought to be very essential.

The multihormonal view of glucose homeostasis states that various hormones such as insulin, glucagon, amylin,

glucagon like peptide-1, glucose-dependent insulinotropic peptide, epinephrine, cortisol and growth hormones regulate glucose metabolism.<sup>2</sup> Along these hormones various neurotransmitters are released in response to nutritional, emotional and environmental changes which maintain blood glucose level but they are still not revealed completely. All these regulating factors are grouped as genetic and epigenetic systems which interact with an equitably complex environmental and behavioral factors.<sup>3</sup> Hence with the advancing genotyping techniques the Genome-wide association studies (GWAS)<sup>4</sup> were done which show a strong interaction between genetic and environmental factors. Genes such as CDKAL1, CDKN2A, CDKN2B, MTNR1B, TCF7L2, KCNJ11, FTO, IRS1, PPARG were associated with T2DM.<sup>5</sup> Here, the major issue in Diabetes biology is to identify the occurrence and prevalence of common genetic variant in different population. In India, due to wide ethnic diversity, Gene analysis among Indian population gave a heterogeneous picture. There was a big difference in the risk allele frequency and pattern of linkage disequilibrium compared to Europeans. The most common genes that have been associated with T2DM in Indian population were

PPARG, KCNJ11, TCF7L2, SLC30A8, HHEX, CDKN2A, IGF2BP2, and CDKAL1.<sup>6,7</sup> Among all, the Transcription Factor 7 Like 2 (TCF7L2) gene show a strong association with T2DM both in Indian and European population.<sup>8-10</sup>

The TCF7L2 gene codes for transcription factor which plays an imperative role in insulin release and regulation of blood glucose homeostasis, cell proliferation and satiety.<sup>11</sup> The transcription factor TCF7L2 initiates transcription of complex Proglucagon gene which are present in  $\alpha$  cells of endocrine pancreas, enteroendocrine L cells of intestine and brain neuronal cells via the Canonical Wnt signaling pathway. Specifically in intestine, in the enteroendocrine L cells of distal ileum the transcription of Proglucagon gene followed by differential tissue specific post translational processing yields Glicentin, Oxyntomodulin, two Glucagon like peptides (GLP-1 and GLP-2) and an intervening peptides 2(IP-2). This is activated by Prohormone Convertase 1(PC1).<sup>12</sup> Among all, incretin hormone GLP-1 regulates-synthesis, processing, secretion of insulin, proliferation and neogenesis of  $\beta$ -cell of pancreas and also protection of  $\beta$ -cells against apoptosis. GLP-1 also maintains the integrity of insulin sensitivity on tissues.<sup>13,14</sup> Many researches state that metabolism of GLP-1 depends on components in diet, blood glucose level and the hormonal signals. This mutual regulation between GLP-1 and glucose metabolism is said as Glucose Competence Concept.<sup>15</sup> Finally insulin release from the readily releasable and immediately releasable pool of primed and docked granules are triggered by the synergistically acting K-ATP channel-dependent and independent signaling pathways. Again these are intrinsically regulated by the Enteroinsular axis via the neural, hormonal and the circulating nutrient metabolites.<sup>16-18</sup> TCF7L2 is found to be the initiating factor for entire cascade of Glucose metabolism.

As determined by Duval et al., (2000), the gene encoding TCF7L2 gene is in high mobility group (HMG) box with 17 exons.<sup>19</sup> By Grant et al., for TCF7L2 gene 228 microsatellite markers were genotyped.<sup>20</sup> The TCF7L2 gene spans ~216kb with approximately 750 single nucleotide polymorphism. From the International HapMap project data, TCF7L2 was found to span several linkage disequilibrium [LD] blocks.<sup>20</sup> The SNPs associated with T2DM forms the largest LD block which spans ~65kb. This LD block begins from middle of the intron 3 and ends at the first seventh of intron 4.<sup>20</sup> Specifically, the two SNPs at rs12255372 and rs7903146 were in strong linkage disequilibrium with Microsatellite DG 10S478. This might lead to altered GLP-1 function and concentration which causes reduced postprandial insulin secretion and enhanced rate of hepatic glucose production.<sup>21,22</sup>

This reflected that the insulin metabolism can be altered by the polymorphisms in TCF7L2 gene leading to inappropriate insulin secretion and action.<sup>13</sup> The polymorphic variants of TCF7L2 gene identified in different population are rs7903146 (C/T), rs7901695 (T/C), rs12255372 (G/T) and rs11196205 (G/C). Amongst, the SNP at rs12255372 and rs7903146 show a strong association with T2DM in many populations all over the world.<sup>22</sup> Hence, we decided to conduct a study to

analyze the association of TCF7L2 gene polymorphism for rs7903146(C/T) and rs12255372(G/T) variants with T2DM for Chennai suburban population in India.

## Materials and Methods

### Study Population

This case-control study was conducted in Govt. Kilpauk Medical College and Hospital (GKM), Chennai by recruiting subjects attending diabetic and general outpatient department. Based on convenient sampling method, the sample size for this study was 44 known T2DM patients under drug treatment and 44 age with gender matched non-diabetic controls from volunteer population. Controls were selected among subjects with their HbA1c value within normal range based on American Diabetic Association-diagnostic criteria.<sup>23</sup> After screening for 53 controls who volunteered for the study, 44 controls were included and 9 subjects were excluded as their HbA1c value were found to be in Diabetic range. Exclusion criteria were subjects with known history of T1DM, other endocrine disorder and pregnant women.

The Institutional Ethics Committee of Govt. Kilpauk Medical College under The TN Dr.MGR Medical University approved the study protocol. After obtaining informed consent from the participants, under aseptic condition 5ml of postprandial venous blood samples were collected. Then 2ml of this blood was transferred to EDTA tube for HbA1c estimation by HPLC method. Remaining 3 ml of blood was transferred to another EDTA tube which was centrifuged at 2500 rpm for 20 minutes. From this buffy coat was extracted carefully and used for DNA extraction using Genomic DNA Minispin Prep kit by HELINI in Genetic laboratory at GKM, Chennai. Later the plasma was separated and stored at -20°C for analysis of Glucose (glucose oxidase-peroxidase method), serum Cholesterol(Cholesterol Esterase-Cholesterol Oxidase CHOD-PAP) and High-density lipoprotein (HDL)-cholesterol (Direct, Enzymatic) in Robonic chemistry auto analyser, at Central biochemistry laboratory, GKM.

### Molecular Genotyping

The DNA extracted from the buffy coat was based on low salt elution method – which is exclusive silica based membrane technology in the form of convenient spin column. Extracted DNA was run in 1.2% agarose gel electrophoresis by comparing with a known molecular weight, 100 bp DNA ladder or Lambda EcoRI/Hind III DNA marker. Concentration of extracted DNA was estimated using UV spectroscopy at 260nm where the absorbance was 0.0066. Then the concentration was calculated using formula:-

1 OD is equivalent to 50µg/ml

Concentration of DNA = absorbance  $\times$  50µg/ml

$\times$  dilution factor = 0.0067  $\times$  50  $\times$  60 = 20 ng/µl

Also the Purity of extracted DNA was assessed by 260/280 ratio and it was found to be > 1.7.

Then the TCF7L2 gene polymorphism for

rs7903146(C/T) was determined by PCR-RFLP technique and for rs12255372(G/T) by Allele specific PCR-ARMS(Amplification Refractory Mutation System) technique in CYBERLAB SMART PCR-PRO thermal cycler.

For rs7903146(C/T) polymorphism, the gene of interest having 578bp size was amplified using the two primers: Forward 5'-CTG TTT CTT GCT TAG TCA CTT TCT G-3' and Reverse 5'-CTT TCA CTA TGT ATT GTT GCC AGT C-3'. PCR done with reaction volume 20 $\mu$ l which contained 10 $\mu$ l of master mix, 2.5 $\mu$ l(10pmols) of each primer and

5 $\mu$ l (100ng) of DNA. Composition of 2X PCR master mix were 10mM of Tris Hcl and 50mM of KCl as buffer, 2mM of MgCl<sub>2</sub>, 200 $\mu$ M of each dNTP's and 1.25 U of Taq polymerase. PCR was done under following condition: initial denaturation at 95°C for 5min, followed by 34 amplification cycles of denaturation at 95°C for 30s, annealing at 56°C for 30s, extension at 72°C for 30s and final extension at 72°C for 9min. A 20 $\mu$ l of the PCR products were digested for 2h at 65°C with 10U of Hpy-CH4III restriction enzyme. Digested gene products were visualized by 3% agarose electrophoresis

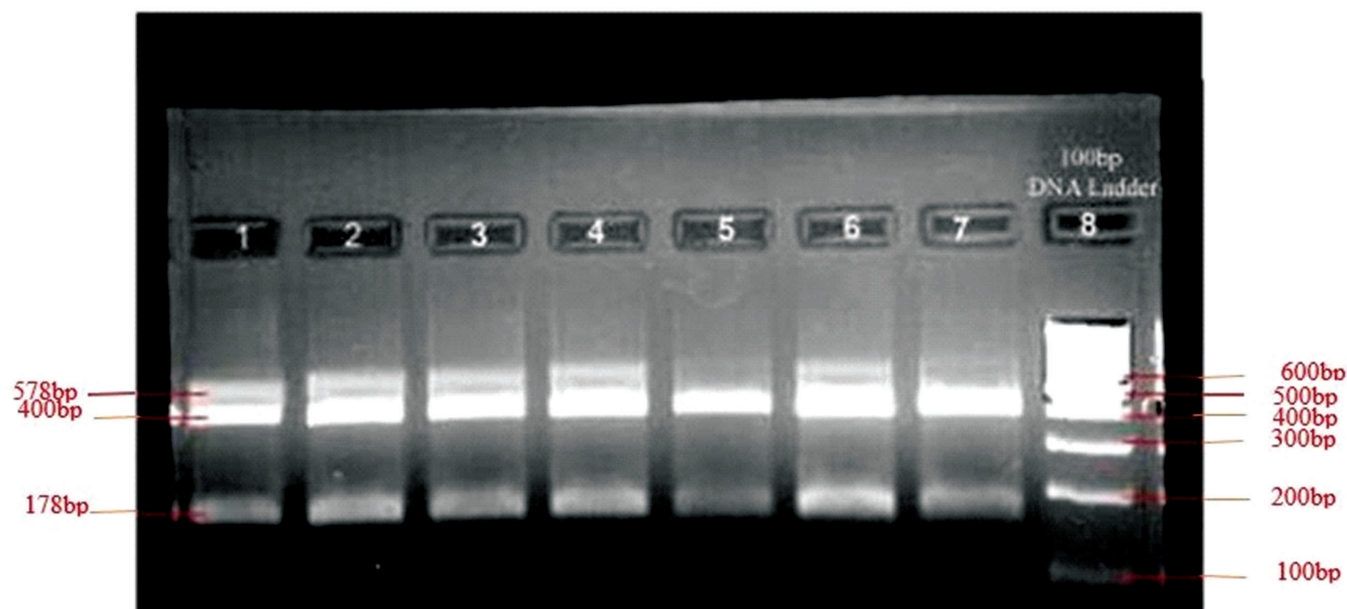


Fig. 1: Restriction Digestion Product by RFLP-rs7903146

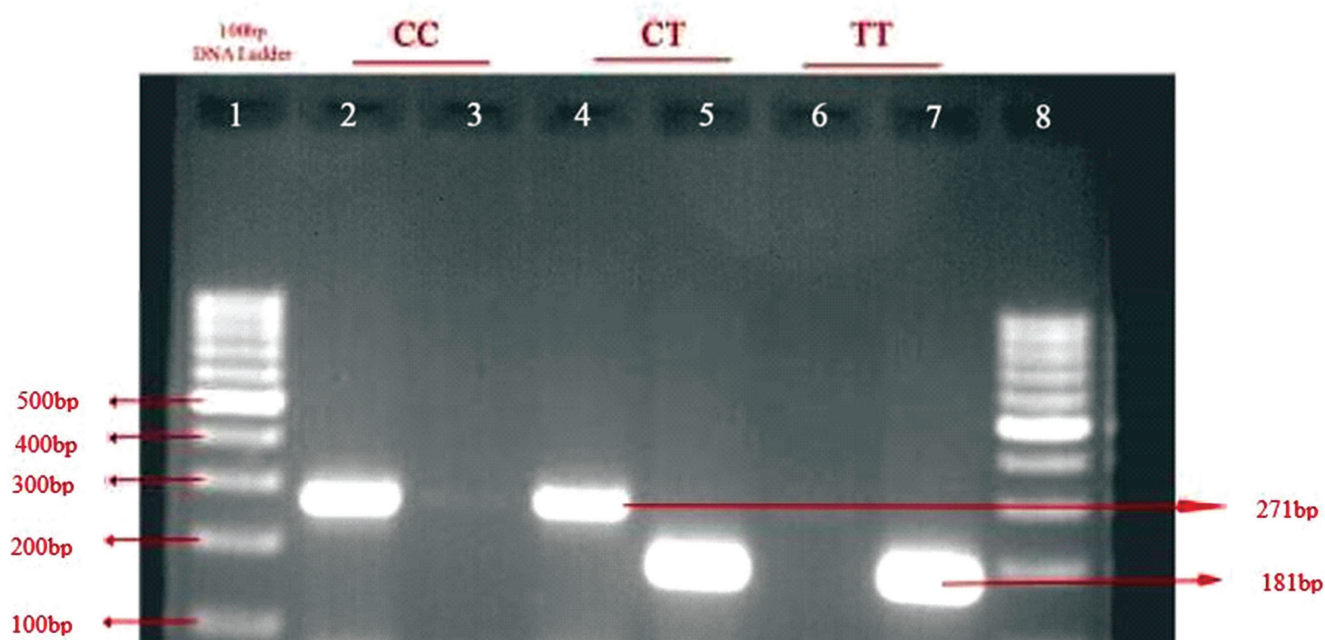


Fig. 2: ARMS- PCR Product 12255372



**Table 1: Anthropometric and Biochemical analysis of the study group**

	Cases (n)	Controls(n)	P value
N	44	44	0.921
Men/Women	20/24	20/24	0.584
Age(yrs)	49.7 (+ 8.5)	45.2 (+ 8.3)	0.921
BMI(kg/m2)	25.5 (+ 4.7)	27.1 (+ 3.3)	0.081(NS)
Waist circumference	103.4 (+ 15.5)	94.5 (+ 8.15)	< 0.001
Bl Glucose (mg/dL)	244.8(+ 85.3)	108.6( + 23.5)	<0.001
HbA1c	8.42 (+ 1.83)	5.21(+ 0.37)	< 0.001
Total Cholesterol (mg/dL)	174.8 (+ 27.1)	169.8 (+ 20.5)	0.325(NS)
HDL Cholesterol (mg/dL)	43.8 (+ 8.77)	44.7 (+ 7.47)	0.611(NS)
Urea (mg/dL)	24.05(+4.62)	25.54(+5.93)	0.190(NA)
Creatinine (mg/dL)	0.862(+0.15)	0.97(+0.16)	0.002(NA)
NA not applicable, NS not significant			

and based on the fragment size of the restriction enzyme digested product, three genotypes were determined: CC genotype with 400 and 178 bp, CT genotype with 578, 400 and 178 bp and TT genotype with 578bp (Fig.1).

For rs12255372(G/T) polymorphism, allele specific ARMS-PCR was done using Forward primer 5'-GAGGCCTGAGTAATTATCAGAATATGATC-3', Reverse primer 5'-AGTCATTTGATGATTGTTTTGTTAATGGC-3' for G allele and Forward primer 5'-CTGCCCAGGAATATCCAGGCAAGAGTT-3', Reverse primer 5'-GAGAGAGTGCCTAAAGACGTGGATTC-T-3' for T allele. The PCR cycles had: 5mins at 95°C, followed by 34 cycles of 1min at 95°C, 1min at 64°C and 1min at 72°C. Final extension was 5min at 72°C. The PCR products (271 bp for G allele and 181 bp for T allele) were identified and analysed on 2% agarose gel stained with ethidium bromide by comparison with a known 100 bp DNA ladder. (Fig. 2)

Finally the genes were visualized under UV illumination and subsequently the images were Gel documented in Life Technologies Gel-doc system. The amplified PCR products were analysed for its accuracy of genotype discrimination by automated sequencer (ShrimpexBSC6616-Applied Biosystems). Two random PCR products were selected and by direct sequencing the results were validated.

### Statistical Analysis

Data was analyzed with SPSS software, version 12.

Comparison between groups were done by student t-test. Genotype and allele frequencies were compared using the  $\chi^2$  statistics. By logistic regression analysis, odds ratio with two tailed p values and 95% confidence intervals(CI) were calculated to assess strength of association. Level of significance for p-value was set at point < 0.05. The frequency of genotypes were tested for Hardy-Weinberg equilibrium.

### Results

The details of Clinical and Biochemical features of the study participants are given in Table 1. The data show that waist circumference show significant difference between cases and controls (p<0.001) rather than Body mass index. Genotype and allele frequencies data for rs7903146 and rs12255372 variant are presented in Table 2 & 3 respectively. The genotype frequency obey Hardy-Weinberg law and are in equilibrium (p value = 0.748). In this study, genotype and allele frequencies between cases and controls yield significant difference in rs7903146(C/T) variant only.

For TCF7L2 rs7903146(C/T) polymorphic variant, the frequency of minor allele(T) was greater in diabetic group (30.7% ) against control group (2.3%) which show that T allele has significantly increased risk for T2DM in this study population. Logistic regression analysis show significantly increased risk for T2DM with minor allele genotype (CT) compared with CC reference genotype with an OR of 0.33 (95% CI 0.07-1.56, p<0.001) (Table 2).

**Table 2: Genotype and Allele frequency of the rs7903146 (C/T) polymorphism and association of this variant with Type 2 Diabetes in the study gro**

Genotype rs7903146 (C/T)	Cases (n = 44)	Controls (n = 44)	Unadjusted OR	(%95 CI)	P value
CT	27 (61.4%)	2 (4.5%)	0.33	(0.07–1.56)	<0.001
CC	17 (38.6%)	42 (95.5%)	Reference		
Frequency of T allele	30.7%	2.3%	0.19	(0.04 – 0.83)	<0.001
Data are presented as mean $\pm$ SD					

**Table 3:** Genotype and Allele frequency of the rs12255372(G/T) polymorphism and association of this variant with type 2 diabetes in the study group

Genotype rs12255372(G/T)	Cases (n = 44)	Controls (n = 44)	Unadjusted OR	(%95 CI)	P value
TT	1(2.3%)	2 (4.5%)	0.67	(0.06 – 7.79)	NS
GT	19(43.2%)	10(22.7%)	2.53	(1.01 – 6.42)	NS
GG	24(54.5%)	32(72.7%)	Reference		
Frequency of T allele	23.9%	15.9%	1.66	(0.78 - 3.53)	NS

Data are presented as mean  $\pm$  SD

For rs12255372(G/T) polymorphic variant analysis did not yield significant difference on genotype and allele frequencies which represent the lack of association between TCF7L2 gene polymorphism at rs12255372 and T2DM in this study population with OR as presented in Table 3.

## Discussion

T2DM is a metabolic disorder with heterogeneous etiology. Many genetic and acquired factors contribute to diabetes where each were also found to have an additive effect on each other. Though genetic factors depend on its pattern of inheritance, their expression is regulated by environmental factors through epigenetic mechanism. This emphasizes that the primary level of prevention for Diabetes starting from lifestyle modification could be a better intervention. Many genes have been identified to be associated with Diabetes. Yet the prevalence of their polymorphism in various ethnic groups has to be evaluated as it has an impact on the expression of genes.<sup>24</sup>

Though many researches proved various candidate genes to be associated with T2DM in different population,<sup>8</sup> recent focus were on the TCF7L2 gene that regulate GLP-1 (incretin hormone) metabolism. Synthesis of GLP-1 is initiated by TCF7L2 factor via Wnt  $\beta$  catenin pathway. TCF7L2 is the only gene with intronic SNP (rs7903146, rs12255372, rs4506565) that showed significant association with T2DM and this has been also been replicated in many population.<sup>25–29</sup> Our study adds to this epidemiological data which specifically pinnacles the genetic determinant of the modern epidemic T2DM.

In our study we have found that among genetically distinct South Indian Chennai population, TCF7L2 gene with polymorphism at rs7903146(C/T) show increased susceptibility to T2DM and rs12255372(G/T) did not find any association. For polymorphism at rs7903146, the polymorphic genotype (CT,TT) frequency is significantly increased in diabetic group (61.4%) compared to control group and it is consistent with study on Indian population (59.1%) and various world population like UK (61.5%), Dutch(60%), Sweden (45.2%). The frequency of T allele was significantly higher in diabetic population (30.7%) which is in accordance with study on south Indian population(33%),western Indian population(37%) and world population like Dutch(34%), Sweden(22%).<sup>9,10,26,28,30</sup>

The polymorphism at rs12255372(G/T) was not

significantly associated with T2DM with a low frequency of risk T allele in diabetic group(24%) which is similar to study on other ethnic population in India. The T allele frequency were found to be (24%) in study by Uma Jyothi et al ,(30%)Chandak et al and (3.6%) in Japanese. This states that TCF7L2 at rs12255372(G/T) has reduced risk for T2DM compared to other sites.<sup>9,30,31</sup> This is in contradiction to study on world western population where the frequency of the risk T allele at rs12255372 is higher compared to other risk alleles in various restriction sites, as in US(33%), Cameroonian (44%), Dutch(34%).<sup>26,32,33</sup> Among all, in the Arab and Japanese population, there is a low frequency of risk T allele at both restriction site - rs7903146, rs12255372 of TCF7L2 gene though this gene polymorphism is significantly associated with T2DM in them.<sup>31,34</sup>

## Conclusion

Our study shows a significant association of TCF7L2 gene polymorphism particularly for the intronic variant rs7903146 with T2DM among urban Chennai population. Previous study stated a mechanism that the alternative splicing sites and the alternative transcription start site in the promoter region led to the synthesis of multiple TCF7L2 protein isoforms and they were found to either activate or repress the Wnt signaling pathway. But the mechanism behind TCF7L2 variant affecting its expression or protein product formation is unclear and also the reason why specifically the impaired action of rs7903146 variant of TCF7L2 gene affect beta cell function is not clearly understood. However, the findings suggest that TCF7L2 genotyping could have valuable clinical application in near future as these genetic variants have been consistently associated with T2DM and it may require a fast and economic assays to meet the needs. The MALDI-TOF mass spectroscopy using SEQUENOM platform and TaqMan SNP genotyping assay are the sophisticated technologies that require expensive dedicated instrumentation and maintenance. Practically every genetic laboratory has standard PCR instruments which is highly desired for genotyping by the routine PCR-RFLP method. Our study have added significance to this allele-specific PCR-ARMS methodology for genotyping rs12255372 variant by this method which was simple and also rapid. The procedure for this method has been detailed. The results were supporting the evidence as given by earlier studies<sup>35</sup> and we also confirmed by gene sequencing on random sample. However in this genetic study

that gave significant and essential results, the small sample size would have been the cause for low statistical power and this can be overcome by doing replicative study on large sample size.

**Conflict of Interest:** None.

## References

1. International Diabetes Federation. *Annual Report* 2016. 2016.
2. Balducci S, Zanuso S, Nicolucci A, Fernando F, Cavallo S, Cardelli P, et al. Glucose Metabolism and Regulation: Beyond Insulin and Glucagon. *Nutr Metab Cardiovasc Dis* 2010;20(8):608–17.
3. DeFronzo RA. From the Triumvirate to the Ominous Octet: A New Paradigm for the Treatment of Type 2 Diabetes Mellitus. *Diabetes* [Internet]. 2009 Apr 1;58(4):773–95. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/16415884>.
4. Frayling TM. Genome-wide association studies provide new insights into type 2 diabetes aetiology. *Nat Rev Genet* [Internet]. 2007 Sep;8(9):657–62. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/16415884>.
5. McCarthy MI. Genomics, type 2 diabetes, and obesity. *N Engl J Med* [Internet]. 2010;363(24):2339–50. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/16415884>.
6. Chauhan G, Spurgeon CJ, Tabassum R, Bhaskar S, Kulkarni SR, Mahajan A, et al. Impact of Common Variants of PPARG, KCNJ11, TCF7L2, SLC30A8, HHEX, CDKN2A, IGF2BP2, and CDKAL1 on the Risk of Type 2 Diabetes in 5,164 Indians. *Diabetes*. 2010;59(8):2068–74.
7. Singh S. The Genetics of Type 2 Diabetes Mellitus: A Review. *J Sci Res banaras hindu Univ* [Internet]. 2011;55:35–48. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/16415884>.
8. Cauchi S, El Achhab Y, Choquet H, Dina C, Kremler F, Weitgasser R, et al. TCF7L2 is reproducibly associated with type 2 diabetes in various ethnic groups: a global meta-analysis. *J Mol Med (Berl)* [Internet]. 2007;85(7):777–82. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/16415884>.
9. Chandak GR, Janipalli CS, Bhaskar S, Kulkarni SR, Mohankrishna P, Hattersley AT, et al. Common variants in the TCF7L2 gene are strongly associated with type 2 diabetes mellitus in the Indian population. *Diabetologia* [Internet]. 2006;50(1):63–7. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/16415884>.
10. Bodhini D, Radha V, Dhar M, Narayani N, Mohan V. The rs12255372(G/T) and rs7903146(C/T) polymorphisms of the TCF7L2 gene are associated with type 2 diabetes mellitus in Asian Indians. *Metabolism* [Internet]. 2007;56(9):1174–8. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/16415884>.
11. Shao W, Wang D, Chiang Y-T, Ip W, Zhu L, Xu F, et al. The Wnt Signaling Pathway Effector TCF7L2 Controls Gut and Brain Proglucagon Gene Expression and Glucose Homeostasis. *Diabetes*. 2013;62(3):789–800.
12. Ugleholdt R, Zhu X, Deacon CF, Ørskov C, Steiner DF, Holst JJ. Impaired Intestinal Proglucagon Processing in Mice Lacking Prohormone Convertase 1. *Endocrinology* [Internet]. 2004;145(3):1349–55. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/16415884>.
13. Xiong X, Shao W, Jin T. New insight into the mechanisms underlying the function of the incretin hormone glucagon-like peptide-1 in pancreatic  $\beta$ -cells. *Islets* [Internet]. 2012;27;4(6):359–65. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/16415884>.
14. Drucker DJ. The biology of incretin hormones. *Cell Metab* [Internet]. 2006;3(3):153–65. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/16415884>.
15. Holz GG, Habener JF. Signal transduction crosstalk in the endocrine system: pancreatic beta-cells and the glucose competence concept. *Trends Biochem Sci* 1992;17(10):388–93.
16. Creutzfeldt W. The incretin concept today. *Diabetologia* 1979;16(2):75–85.
17. Unger RH, Eisentraut AM. Entero-insular axis. *Arch Intern Med* 1969;123(3):261–6.
18. Morgan LM, Flatt PR, Marks V, Morgan. Nutrient regulation of the enteroinsular axis and insulin secretion. *Nutr Res Rev* 1988;1(1):79–97.
19. Hansson O, Zhou Y, Renström E, Osmark P. Molecular Function of TCF7L2: Consequences of TCF7L2 Splicing for Molecular Function and Risk for Type 2 Diabetes. *Curr Diab Rep* 2010;10(6):444–51.
20. Grant SFA, Thorleifsson G, Reynisdottir I, Benediktsson R, Manolescu A, Sainz J, et al. Variant of transcription factor 7-like 2 (TCF7L2) gene confers risk of type 2 diabetes. *Nat Genet* [Internet]. 2006;38(3):320–3. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/16415884>.
21. Jin T. Mechanisms underlying proglucagon gene expression. *J Endocrinol* 2008;198(1):17–28.
22. Gupta V, Khadgawat R, Saraswathy KN, Sachdeva MP, Kalla AK. Emergence of TCF7L2 as a Most Promising Gene in Predisposition of Diabetes Type II. *Int J Hum Genet* [Internet]. 2008;8(1–2):199–215. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/16415884>.
23. Classification I. Standards of Medical Care in Diabetes-2014. *Diabetes Care* 2014;37(Supplement\_1):S14–80.
24. International T, Consortium H. A haplotype map of the human genome. *Nature* 2005;437(7063):1299–320.
25. Groves CJ, Zeggini E, Minton J, Frayling TM, Weedon MN, Rayner NW, 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* [Internet]. 2006;55(9):2640–4. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/16415884>.
26. van Vliet-Ostapchouk J V., Shiri-Sverdlov R, Zhernakova A, Strengman E, van Haeften TW, Hofker MH, et al. Association of variants of transcription factor 7-like 2 (TCF7L2) with susceptibility to type 2 diabetes in the Dutch Breda cohort. *Diabetologia* [Internet]. 2007;50(1):59–62. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/16415884>.
27. Scott LJ, Bonnycastle LL, Willer CJ, Sprau AG, Jackson AU, Narisu N, et al. Association of Transcription Factor 7-Like 2 (TCF7L2) Variants With Type 2 Diabetes in a Finnish Sample. *Diabetes* [Internet]. 2006;55(9):2649–53. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/16415884>.
28. Mayans S, Lackovic K, Lindgren P, Ruikka K, Ågren Å, Eliasson M, et al. TCF7L2 polymorphisms are associated with type 2 diabetes in northern Sweden. *Eur J Hum Genet* [Internet]. 2007;15(3):342–6. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/16415884>.

29. Cauchi S, Meyre D, Dina C, Choquet H, Samson C, Gallina S, et al. Transcription Factor TCF7L2 Genetic Study in the French Population: Expression in Human -Cells and Adipose Tissue and Strong Association With Type 2 Diabetes. *Diabetes* [Internet]. 2006;55(10):2903–8. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/16415884>.
30. Uma Jyothi K, Jayaraj M, Subburaj KS, Prasad KJ, Kumuda I, Lakshmi V, et al. Association of TCF7L2 Gene Polymorphisms with T2DM in the Population of Hyderabad, India. Huang Q, editor. *PLoS One* [Internet]. 2013;8(4):e60212. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/16415884>.
31. Hayashi T, Iwamoto Y, Kaku K, Hirose H, Maeda S. Replication study for the association of TCF7L2 with susceptibility to type 2 diabetes in a Japanese population. *Diabetologia* [Internet]. 2007;50(5):980–4. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/16415884>.
32. Zhang C, Qi L, Hunter DJ, Meigs JB, Manson JE, van Dam RM, et al. Variant of Transcription Factor 7-Like 2 (TCF7L2 ) Gene and the Risk of Type 2 Diabetes in Large Cohorts of U.S. Women and Men. *Diabetes* [Internet]. 2006;55(9):2645–8. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/16415884>.
33. Nanfa D, Sobngwi E, Atogho-Tiedeu B, Noubiap JJN, Donfack OS, Mofo EPM, et al. Association between the TCF7L2 rs12255372 (G/T) gene polymorphism and type 2 diabetes mellitus in a Cameroonian population: a pilot study. *Clin Transl Med* [Internet]. 2015;4(1):17. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/16415884>.
34. Alsmadi O, Al-Rubeaan K, Mohamed G, Alkayal F, Al-Saud H, Al-Saud NA, et al. Weak or no association of TCF7L2 variants with Type 2 diabetes risk in an Arab population. *BMC Med Genet* [Internet]. 2008;9(1):72. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/16415884>.
35. Dutra LAS, Costa PGG, Velasco LFR, Amato AA, Barra GB. Allele-specific PCR assay to genotype SNP rs7903146 in TCF7L2 gene for rapid screening of diabetes susceptibility. *Arq Bras Endocrinol Metabol* 2008;52(8):1362–6.

**How to cite this article:** Devi BL, Meera V, Nagendran R, Lalitha R, Komala G. Association of variants of the TCF7L2 gene at rs7903146(C/T) and rs12255372(G/T) with type 2 diabetes mellitus in Chennai suburban population. *Int J Clin Biochem Res* 2019;6(2):190-6.