Suez Canal University Medical Journal Vol. 19 (1), 2016
Pages 29-38

Association of RS 7903146 (C/T) Single Nucleotide Polymorphism at Transcription Factor 7 Like 2 Gene with Type 2 Diabetes Mellitus in Egyptian Patients

Nahed Y. Abd El Razek[1*], Neveen R. Wissa[1], Hassnaa M. Nassar[1], Seham A. Omar[2]

Departments of [1]Clinical Pathology, and [2]Internal Medicine, Faculty of Medicine, Suez Canal University

Abstract

Background: **Many loci were found to be associated with type 2 diabetes mellitus (T2DM) risk as single nucleotide polymorphism (SNP) at the transcription factor 7-like 2 gene (TCF7L2) locus on chromosome 10q (rs7903146) [C/T].** ***Aim:*** **Estimation of the association of this gene polymorphism with T2DM and its complications in Egyptian population.** ***Patients and Methods:*** **This study was done, using 90 Egyptian T2DM patients and 100 controls. This polymorphism was genotyped by conventional PCR. Measurement of blood glucose, glycated hemoglobin (HbA_{1c}), lipid profile, and microalbuminuria were performed for the study subjects using standard methods. Body Mass index and fundus examination for detection of diabetic retinopathy were also done.** ***Results:*** **The genotype and allele frequencies in TCF7L2 rs7903146 were nearly the same in the patient and control groups (P > 0.05).Odds Ratio for the high risk allele (T) of (rs7903146) was (OR) = 0.97 with 95% confidence interval (CI) from 0.61 to 1.54 with the P = 0.9.** ***Conclusion:*** **These data suggest that the TCF7L2 SNP rs7903146 may not significantly contribute to T2DM susceptibility in Egyptian population.**

Key words: Allele specific PCR, Wnt signaling pathway, Suez Canal University Hospital

Introduction

Type 2 diabetes mellitus (T2DM) is a heterogeneous group of disorders usually characterized by the incapability of pancreatic β cells to increase insulin secretion to compensate the insulin resistance in peripheral tissues[(1).] T2DM is a multi-factorial disease, and the susceptibility to it is determined by several genetic and environmental factors [(2)]. A large scale genome wide association scan (GWAS) study reported that some single nucleotide polymorphisms (SNPs) in the TCF7L2 gene were strongly associated with risk to T2DM in an Iceland case-control sample[(3)]. Afterwards, this association has been replicated by numerous groups among different ethnicities[(4)]. Amongst the TCF7L2 variants, one intronic SNP (rs7903146) (C/T) variant

**Corresponding Author: nahedyoussef2003@yahoo.com*

is closely associated with T2DM[4-9]. The TCF7L2 gene encodes a transcription factor involved in the Wnt signaling pathway, which plays an important role in pancreatic islet development and Adipogenesis[10]. TCF7L2 forms heterodimers with b-catenin, inducing the expression of various genes, including the insulinotropic hormone glucagon-like peptide 1 (GLP-1) gene, the insulin gene, and other genes that encode proteins involved in processing and exocytose of insulin granules[11-13]. As GLP-1 and insulin play a key role in blood glucose homeostasis, it was hypothesized that TCF7L2 variants may modify T2DM susceptibility by indirectly reducing GLP-1 secretion from entero-endocrine cells[14]. On the other hand, as the Wnt pathway seems to be important for pancreas development during embryonic growth, it is also possible that the β-cell mass, pancreatic β cell development and/or β-cell function are also affected by this pathway[15]. However, the exact molecular mechanism underlying the association of TCF7L2 polymorphisms with T2DM remains to be enlightened[16,17]. Because the frequency of the TCF7L2 rs7903146 T allele has been shown variable among different populations[18-24], we investigated, in the present study, the potential association of the TCF7L2 rs7903146 (C/T) polymorphism with T2DM and some of its complications, in Egyptian diabeticsubjects.

Patients and Methods

Patients

A total of 190 unrelated subjects were enrolled in this case-control study. The diabetic sample comprised 90 T2DM patients.T2DM was diagnosed according to the American Diabetes Association criteria[25]. The non-diabetic group comprised 100 healthy volunteers attending the blood donation facility at Suez Canal University hospital. None of these subjects reported presence of diabetes or a family history of this disease. All study subjects underwent physical examination and laboratory evaluations. BMI was calculated as weight (kg)/height square (meters). Serum and blood from study subjects were taken after a 10 hours of fasting for laboratory analyses[26]. Plasma glucose levels were determined using the glucose oxidase method. HbA1c measurements were performed by Turbidimetric inhibition immunoassay (TINA)[27]. Total plasma cholesterol, HDL cholesterol and triglycerides were assayed using enzymatic methods. LDL cholesterol were calculated according to Friedewald Formula. The information obtained from the study did not influence patients' diagnosis or treatment. The study protocol was approved by the Ethic Committee in Research from Suez Canal University Hospital and all patients and non-diabetic subjects provided written informed consent.

Genotyping

DNA was extracted from peripheral blood leucocytes of EDTA anti-coagulated blood using QIAamp® DNA Mini and Blood Mini Handbook 2010. TCF7L2 rs7903146 (C/T) SNP was genotyped using primers (metabion, Germany) according to the manufacturer's protocol. The allele-specific forward primers (rs7903146 C or rs7903146 T) and the reverse primer

(rs7903146 R) were combined in two parallel PCR reactions as previously reported[28]. Sequences of primers were: GAACAATTAGAGAGCTAAGCACT TTTTAGAAAC (Forward primer of allele C), GAACAATTAGAGAGCTAAGCA CTTTTTAGAGAT (Forward primer of allele T), and GATGAAATGTAGCAGT GAAGTC (Common reverse primer R). Reactions were conducted in 36-well plates. For each sample, two PCR reactions were run in parallel, each containing 5 µg of genomic DNA, 4 µl of 5x HOT FIREPol® Blend Master Mix Ready to Load, 0.5 µl Forward primer (10 pmol/µl), 0.5 µl Reverse primer (10 pmol/µl), and Up to 20 µl H2O. Amplification was performed on real time thermal cycler and the cycling conditions were as follows: initial denaturation for 3 min at 94 °C; followed by 32 cycles of 1 min at 50 °C, and 1 min at 72 °C; and final extension for 5 min at 72 °C. The PCR products were analyzed by electrophoresis on 3% Tris-borate-EDTA/ethidium bromide agarose gels, visualized under ultraviolet illumination and the presence of a 205-bp band in PCR C or T indicated the presence of the allele. A sample was considered negative for a particular allele when the amplicon was absent.

Statistical Analyses

The magnitude of associations of the TCF7L2 rs7903146 SNP with T2DM were estimated using odds ratio (OR) with 95% confidence interval (CI). Regression analyses were performed to assess the independent association of this SNP with T2DM complications. Results for which the P value was under 0.05 were considered statistically significant. These statistical analyses were performed using SPSS version 16.0 *(SPSS, Chicago, IL).*

Results

The diabetic sample comprised 90 T2DM patients. The main characteristics of the T2DM patients were: mean age was ($48.28^{\pm}14.04$) years, mean glycated hemoglobin (HbA1c) was ($8.92^{\pm}2.22\%$), and mean body mass index (BMI) was ($31.4^{\pm}4.69$) kg/m2 (table 1). Males comprised (24.5%) of the sample. The non-diabetic group comprised 100 healthy volunteers, mean age ($46.12^{\pm}14.30$) years; males (25%). Table 1 summarizes the clinical and laboratory data for T2DM patients according to the different genotypes of the rs7903146 SNP. There were significant differences among the three rs7903146 SNP genotypes in terms of total cholesterol, LDL cholesterol, HbA1c, Fasting blood glucose, postprandial blood glucose, creatinine albumin ratio, and diabetic retinopathy, but there were no significant differences in BMI, triglycerides, and HDL cholesterol. A representation of rs7903146 C/T Genotype by allele-specific PCR is found in figure 1. For each sample, two PCR reaction were performed, one with primes C and R (PCR C) and a second with primers T and R (PCR T), and run side by side on an agarose gel. A 205-bp band indicated the presence of the allele; amplification failure indicated the absence of the allele. In the gel, the first lane shows the product from the size-standard sample; for the other lanes, C and T indicate the product from PCR C

and PCR T, respectively, amplified from negative control (water) and from selected DNA sample of each possible genotypes [1 (CC), 2 (CT) and 3 (TT)].

Table 1: Clinical and laboratory characteristics of patients and controls

	patients	Control	P value
BMI (kg/m^2)	31.4±4.6	23.98 ±3.59	0*
Cho (mg/dl)	207.51±46.54	151.91±15.91	0*
TG (mg/dl)	157.91±81.25	61.44±17.76	0*
HDL (mg/dl)	44.84±13.2	53.78±13.19	0*
LDL (mg/dl)	130.87± 39.12	80.84±21.94	0*
FBS (mg/dl)	185.93± 84.49	85.72±4.19	0*
PP (mg/dl)	286.59±108.5	114.82±5.76	0*
HbA1C (%)	8.92±2.22	5.12±0.23	0*
Alb/ Cr mg/mg	429.72±236.4	19.66±5.26	0*
Retinopathy	50(55.5%	13 (13%)	0*

* P value significant

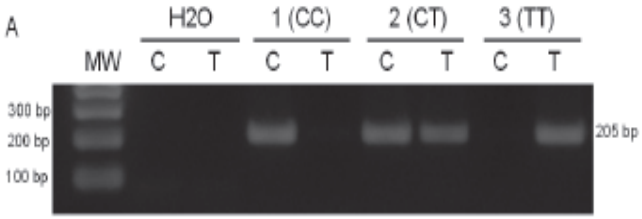

Figure 1: A representation of the genotyping by allele specific PCR.

The patients and controls genotypes were compared regarding TCF7L2 gene polymorphism rs7903146 and divided into 3 subgroups according to their genotype, the first group was homozygous for the low risk allele (C) and called (C/C), the second group was homozygous for the high risk allele (T) and called (T/T), and the third group was heterozygous for both alleles (C) and (T) and called (C/T). Genotype frequencies of the rs7903146 SNP were insignificantly associated with T2DM (Table 2). The T allele was insignificantly associated with risk for T2DM under a dominant model of inheritance. (P=0.9). Moreover, homozygosis for the T allele was associated with a higher risk for T2DM (P = 0.8) than the presence of only one copy of this allele (P=0.16). In the diabetic patients, There was no statistically significant difference in BMI between the groups (P>0.05), but statistically significant difference was found on assessment of retinopathy by fundus examination between groups, (P=0.001). Regarding lipid profile, There was statistically significant difference in cholesterol, and LDL levels between subgroups (p<0.05), being higher in the patients who were homozygous for the T allele of (rs7903146). But there were no significant differences in the triglycerides and HDL levels between subgroups (p >0.05). Regarding glucose levels,

There were statistically significant difference in FBS, PP, and HbA1c levels between subgroups, being higher in the patients who were homozygous for the (T) allele of (rs7903146) (P<0.05). There was statistically significant difference in microalbumin creatinine ratio levels between subgroups (P<0.05), being also higher in the patients who were homozygous for the (T) allele of (rs7903146).

Table 2: Genotype and allele frequencies of rs7903146 (C/T) SNP in T2DM patients and non-diabetic subjects

	Patients N =90	Control N =100	OR (95% CI)	P value
Genotype				
CC	25 (27.78%)	21 (21%)	1	
CT	41 (45.56%)	57 (57%)	0.6 (0.2-1.2)	0.1
TT	24 (26.67%)	22 (22 %)	0.9 (0.4-2.07)	0.8
Allele				0.9
C	0.51	0.49	1	
T	0.49	0.51	0.9 (0.6-1.5)	
Dominant				0.2
CC	25 (27.78%)	21 (21%)	1	
TT	65 (72.23%)	79 (79%)	0.6 (0.3 - 1.3)	

Data are presented as number (%) or proportion

Table 3: Clinical and laboratory characteristics of T2DM patients, broken down by presence of different TCF7L2 rs7903146 (C/T) genotypes

	TCF7L2 rs7903146 (C/T) genotypes			P value
	CC	CT	TT	
Cho (mg/dl)	200.5±53	199.9 ±44.7	227.75±37.03	0.007
TG (mg/dl)	147.8±76.7	151.0±75	180.13±94.31	0.46
HDL (mg/dl)	43.2±13.14	45.76±12.6	45±14.58	0.798
LDL (mg/dl)	130.1±50	123.0±34.4	145.04±29.18	0.014
FBG (mg/dl)	156.7±76.9	177.8±81.8	230.08±81.91	0.003
PP (mg/dl)	251±106.2	270±107.6	351.42±87.37	0.001
HbA1C (%)	7.94±2.08	8.74±1.86	10.24±2.37	0.003
Alb/Cr mg/mg	376±202.2	388.2±232	556.4±237.78	0.008
BMI (kg/m^2)	32.04±5.14	30.9±5.22	31.58±3.01	0.379
Diabetic retinopathy	13 (52%)	16 (39%)	21 (87.5%)	0.001

Data are mean±SD, or %. Cho: cholesterol; TG: triglycerides; BMI: body mass index; FPG: fasting plasma glucose; PP: post prandial blood glucose; HbA1c: glycated hemoglobin; T2DM: type 2 diabetes mellitus; Alb/Cr: albumin creatinine ratio. P ≤ 0.05 is significant.

Discussion

T2DM is a multi-factorial disease, and its susceptibility is determined by several genetic and environmental factors. SNPs at TCF7L2 gene locus on hromosome 10q specially (rs12255372) [G/T] and (rs7903146) [C/T] have been

found to be associated with T2DM risk in various populations and ethnic groups[29]. The rs7903146 T allele is probably the best marker to evaluate the effect of this gene on T2DM risk. This allele is thought to increase the risk of T2DM in different populations including Europeans [30], Japanese and Indians[31], Latin Americans [32] and West Africans [33]. In this study, the patients were divided into three sub groups` according to their genotypes, homozygous for the (C) allele or (T) allele or heterozygous (CT) for (rs7903146) to determine the association of the three genotypes of this gene with other laboratory and anthropometric measures in the Egyptian population. In the present study, there was no statistically significant difference in BMI between patients` subgroups (p >0.05). This was consistent with on Arabs[34] and Algerian[35] populations respectively, in contrast, Phillips et al., 2011, reported that the risk allele (T) was associated with higher BMI on Americans[36]. This controversy might be due to the high prevalence of obesity among Arabs especially Egyptians[37] and also the difference in diet composition[38] that might influence the genetic effect of TCF7L2 polymorphisms[39]. Also the (T) allele homozygous patients had significantly higher serum cholesterol and LDL than the (CT) and (CC) subgroups in this study, In consistence with Sanghera, et al. (2008) who suggested a strong possibility of indirect contribution of this gene in affecting insulin resistance by elevating cholesterol levels which could be independent of obesity in India[40]. Serum fasting, postprandial glucose and HbA1c levels showed statistically significant difference between patients` subgroups being higher in the (T) allele carriers in the present study. These results were supported by Song et al, 2012 meta-analysis [41] who quantified the association of the rs7903146 SNP with measures of beta-cell function among 35,052 non-diabetic subjects from 31 studies. They reported that T allele carriers had significantly higher fasting glucose and 2h post-load glucose levels when compared to C/C homozygous. When comparison was done between patients` sub groups, microalbuminuria level was seen to be statistically significantly higher in the (T) allele carriers. This was in agreement with Sale et al, 2007[42], who reported the association of this gene SNP with diabetic nephropathy (DN) as indicated by persistent urine albuminuria (>300 mg/mg creatinine) in two consecutive measurements. In the present study, Retinopathy was found mainly in (T) allele carriers as 37 out of 50 cases of retinopathy carried the (T) high risk allele. In agreement with Melzer et al 2006[43], and Taís et al., 2014[44] who reported that the (T) allele carriers were more likely to have microvascular complication as poor renal function and retinopathy. Also, Jing et al., 2013[45] reported that TCF7L2, a key component of the Wnt-signaling pathway, plays an important role in pathological neo-vascularization. This is in contrast to Buchbinder et al.(2008)[46] who reported that there is no influence of this gene in the pathogenesis of diabetes-induced microvascular complications as nephropathy and retinopathy and it is mainly related to hyperglycemia. Us-

ing the CC genotype as a reference[47], the Odds ratio (OR) analysis showed that the C/T, T/T, and C/T+T/T genotypes were not associated with an increased risk for T2DM in the population studied (C/T: OR =0.6, 95%CI = 0.29 – 1.22, P = 0.16); (T/T: OR =0.91, 95%CI = 0.4 - 2.07, P = 0.83); (C/T+T/T: OR = 0.7, 95%CI = 0.35 – 1.34, P = 0.28). These data suggested that the TCF7L2 SNP rs7903146 might not contribute to T2DM susceptibility in this population. In agreement with Alsmadi et al., 2008[34], who reported in his study on Saudi Arabian population, that the rs7903146T allele frequency of the cases (0.415) was not different from that observed in the controls (0.4). (OR= 1.04, 95%CI= 0.86–1.27, P =0.6). The CT genotype frequency of the cases he studied, was 0.485 compared to 0.468 among the controls, the difference was not significant when compared to the CC reference genotype (P= 0.57). TT genotype frequency of both cases and controls was also compared to the CC reference genotype and resulted in a p value of 0.757, and an OR of 1.065 with a 95% CI of (0.71–1.59). This indicated no difference between the cases and controls with respect to CT and TT genotypes in his study.

Conclusion

The TCF7L2 SNP rs7903146 may not significantly contribute to T2DM susceptibility in this population. However, these results may be due to the small sample size of subjects and it cannot rule out an effect of other SNPs in this gene in diabetics.

References

1. Stumvoll M, Goldstein B, van Haeften T. Type 2 diabetes: principles of pathogenesis and therapy. Lancet. (2005); 365(9467):1333-1346.
2. Vimaleswaran K, Loos RJ Progress in the genetics of common obesity and type 2 diabetes. Expert Rev Mol Med. (2010); 12:e7.
3. Grant S, 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. (2006); 38(3):320-323.
4. Florez J. The new type 2 diabetes gene TCF7L2. Curr Opin Clin Nutr Metab Care. (2007);10 (4):391-396.
5. Tong Y, Lin Y, Zhang Y, Yang J, Liu H, and Zhang B. Association between TCF7L2 gene polymorphisms and susceptibility to type 2 diabetes mellitus: a large Human Genome Epidemiology (HuGE) review and meta-analysis. BMC Med Genet. (2009); 10:15.
6. Peng S, Zhu Y, Lü B, Xu F, Li X, Lai M. TCF7L2 gene polymorphisms and type 2 diabetes risk: a comprehensive and updated meta-analysis involving 121, 174 subjects. Mutagenesis. 2013; 28(1):25-37.
7. Dou H, Ma E, Yin L, Jin Y, Wang H. The association between gene polymorphism of TCF7L2 and type 2 diabetes in Chinese Han population a meta-analysis. PLoS One. (2013); 8(3):e59495.
8. Wang J, Hu F, Feng T, Zhao J, Yin L, Li L, et al. Meta-analysis of associations between TCF7L2 polymorphisms and risk of type 2 diabetes mellitus in the Chinese population. BMC Med Genet. (2013); 14:8.

9. Ip W, Chiang Y, Jin T. The involvement of the wnt signaling pathway and TCF7L2 in diabetes mellitus: The current understanding, dispute, and perspective. Cell Biosci. (2012); 2(1):28.
10. Prestwich T, Macdougald O. Wnt/β-catenin signaling in adipogenesis and metabolism. Curr Opin Cell Biol. (2007); 19(6):612-617.
11. da Silva Xavier G, Loder M, McDonald A, Tarasov A, Carzaniga R, Kronen-berger K, et al. TCF7L2 regulates late events in insulin secretion from pancreatic islet β-cells. Diabetes. (2009); 58(4):894-905.
12. Loder M, da Silva Xavier G, McDonald A, Rutter G. TCF7L2 controls insulin gene expression and insulin secretion in mature pancreatic β-cells. Biochem Soc Trans. (2008); 36(Pt 3):357-359.
13. Yi F, Brubaker P, Jin T. TCF-4 mediates cell type-specific regulation of proglucagon gene expression by β-catenin and glycogen synthase kinase-3β. J Biol Chem. (2005); 280(2):1457-1464.
14. Smith U. TCF7L2 and type 2 diabetes--we WNT to know. Diabetologia. (2007); 50(1):5-7.
15. Weedon MN. The importance of TCF7L2. Diabetic Medicine. (2007); 24(10):1062-1066.
16. Schfer S, Machicao F, Fritsche A, Hring H, Kantartzis K. New type 2 diabetes risk genes provide new insights in insulin secretion mechanisms. Diabetes Res Clin Pract. (2011); 93 Suppl 1:S9-24.
17. Ezzidi I, Mtiraoui N, Cauchi S, Vaillant E, Dechaume A, Chaieb M, et al. Contribution of type 2 diabetes associated loci in the Arabic population from Tunisia: a case-control study. BMC Med Genet. (2009); 10:33.
18. Gonzalez-Sanchez J, Martinez-Larrad M, Zabena C, Perez-Barba M, Serrano-Rios M. Association of variants of the TCF7L2 gene with increases in the risk of type 2 diabetes and the proinsulin: insulin ratio in the Spanish population. Diabetologia. (2008); 51(11):1993-1997.
19. 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 β-cells and adipose tissue and strong association with type 2 diabetes. Diabetes. (2006); 55(10):2903-2908.
20. Wen J, Ronn T, Olsson A, Yang Z, Lu B, Du Y, et al. Investigation of type 2 diabetes risk alleles support CDKN2A/B, CDKAL1, and TCF7L2 as susceptibility genes in a Han Chinese cohort. PLoS One. (2010); 5(2):e9153.
21. Chang Y, Chang T, Jiang Y, Kuo S, Lee K, Chiu K, 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.
22. Franco L, Crispim F, Pereira AC, Moises R. Variants of transcription factor 7-like 2 (TCF7L2) gene and incident glucose intolerance in Japanese-Brazilians. Braz J Med Biol Res. (2011); 44(3):240-244.
23. Barra G, Dutra L, Watanabe S, Costa P, Cruz P, Azevedo M, et al. Association of the rs7903146 single nucleotide polymorphism at the Transcription Factor 7-like 2 (TCF7L2) locus with type 2 diabetes in Brazilian subjects. Arq Bras Endocrinol Metabol. (2012); 56(8):479-484.
24. Canani L, Capp C, Ng D, Choo S, Maia A, Nabinger G, et al. The fatty acid-binding protein-2 A54T polymor-

phism is associated with renal disease in patients with type 2 diabetes. Diabetes. (2005); 54(11):3326-3330.
25. American Diabetes Association. Diagnosis and Classification of Diabetes Mellitus. Diabetes Care. (2013); 35(Suppl. 1):S64-S71.
26. Bouças A, Brondani L, Souza B, Lemos N, de Oliveira F, Canani L, et al. The A allele of the rs1990760 polymorphism in the IFIH1 gene is associated with protection for arterial hypertension in type 1 diabetic patients and with expression of this gene in human mononuclear cells. PLoS One. (2013); 8(12): e 83451.
27. Vesna S, Lidija B, Elizabeta F. Validation of methods performance for routine biochemistry analytes at Cobas 6000 analyzer series module c501. Biochem Med. (2011); 21(2):182-190.
28. Ludmila A, Patrícia G, Lara F, Angélica A, Gustavo B. Allele-Specific PCR Assay to Genotype SNP rs7903146 in TCF7L2 Gene for Rapid Screening of Diabetes Susceptibility. Arq Bras Endocrinol Metab (2008); 52:8
29. Grant S, 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, (2006); 38(3):320-323.
30. Zeggini E., McCarthy M. TCF7L2the biggest story in diabetes genetics since HLA? Diabetologia. (2007); 50(1):1-4.
31. Chandak G, Janipalli C, Bhaskar S, Kulkarni S, Mohankrishna P, Hattersley A. Common variants in the TCF7L2 gene are strongly associated with type 2 diabetes mellitus in the Indian population. Diabetologia. (2007); (50):63-67
32. Lehman D, Hunt K, Leach R, Hamlington J, Arya R, Abboud, H, Stern M. Haplotypes of transcription factor 7–like 2 (TCF7L2) genes and its upstream region are associated with type 2 diabetes and age of onset in Mexican Americans. Diabetes, (2007); 56 (2):389-393
33. Agnar H, Snæbjörn P,Gudmar T, Struan F, Valur E, Steinunn G, Kári S. Refining the impact of TCF7L2 gene variants on type 2 diabetes and adaptive evolution. Nat Genet. (2007); 39:218–225
34. Alsmadi O, Khalid R, Gamal M, Fadi k, Haya S, Nouran S, Brian F. Weak or no association of TCF7L2 variants with Type 2 diabetes risk in an Arab population. BMC Med Genet. (2008); 9:72.
35. Hadjira O, Sounnia M, Sarah A, Imane H, Djabaria N, Houssam B, Aline M. The TCF7L2 rs7903146 polymorphism, dietary intakes and type 2 diabetes risk in an Algerian population. BMC Genet (2014); 15:134.
36. Phillips C. , Goumidi L, Bertrais S, Field M, McManus R, Hercberg S, Roche H. Dietary saturated fat, gender and genetic variation at the TCF7L2 locus predict the development of metabolic syndrome. J Nutr Biochem (2011); 23(3):239–244
37. Mohammad B, Ismail L. Review Article Type II Diabetes Mellitus in Arabic-Speaking Countries, Int J Endocrinol, vol. (2012); 1-11
38. Karamanos B, Thanopoulou A, Angelico F, Assaad K., Barbato A, Del Ben M, Tenconi M. Nutritional habits in the Mediterranean basin. The macronutrient composition of diet and its relation with the traditional Mediterranean diet. Multi-centre study of the Mediterranean Group for the Study of Diabetes (MGSD).

Eur J Clin Nutr (2002); 56(10):983–991
39. Florez J, Jablonski A, Bayley N, Pollin T., de Bakker P, Shuldiner A, Altshuler D. TCF7L2 polymorphisms and progression to diabetes in the Diabetes Prevention Program. N Engl J Med. (2006); 355(3):241-250.
40. Sanghera D, Nath S, Ortega L, Gambarelli M, Kim-Howard X, Kamboh M. TCF7L2 polymorphisms are associated with type 2 diabetes in KhatriSikhsfrom North India: genetic variation affects lipid levels. Annals Hum Genet, (2008);72 (Pt4): 499– 509.
41. Song Y, Yeung E, Liu A, Vanderweelem T, Chen L, Lu C. Pancreatic beta-cell function and type 2 diabetes risk: quantify the causal effect using a Mendelian randomization approach based on meta-analyses. Hum Mol Genet.(2012); 21(22):5010-5018.
42. Sale M, Smith S, Mychaleckyj J, Keene K, Langefeld C, Leak T, Freedman B. Variants of the transcription factor 7-like 2 (TCF7L2) gene are associated with type 2 diabetes in an African-American population enriched for nephropathy. Diabetes. (2007); 56:2638–2642
43. Melzer D, Murray A, Hurst A , Weedon M, Bandinelli S, Anna M,Timothy M. Effects of the diabetes linked TCF7L2 polymorphism in a representative older population. BMC Med (2006); 4:34.
44. Taís S, Guilherme C, Jakeline R, Lavínia A, Luís H, Daisy C. The TCF7L2 rs7903146 (C/T) polymorphism is associated with risk to type 2 diabetes mellitus in Southern-Brazil. Arq Bras Endocrinol Metab (2014):, 58:59
45. Jing L, Ling Z, Aaron Y, Xiaohui Z, Jin Z, Jiagang Z, Kang Z. TCF7L2 Variation and Proliferative Diabetic Retinopathy. Diabetes.(2013); 62(7):2613–2617
46. Buchbinder S, Rudofsky G, Humpert P, Schilling T, Zorn M, Bierhaus A, Nawroth P. The DG10S478 variant in the TCF7L2 gene is not associated with microvascular complications in type 2 diabetes. Exp Clin Endocrinol Diabetes (2008); 116 (4): 211-214.
47. Barros C, Araujo-Neto A, Lopes T, Barros M, Motta F, Canalle R, Pinto G. Association of the rs7903146 and rs12255372 polymorphisms in the TCF7L2 gene with type 2 diabetes in a population from northeastern Brazil. Genet Mol Res (2014); 13(3):7889-7898.