# *TCF7L2* Gene Polymorphisms and Susceptibility to Type 2 Diabetes Mellitus, A Pilot Study

## Iman Mandour[1], Rania Darwish[1], Randa Fayez[2], Mervat Naguib[2] and Sarah El-Sayegh[1]

[1]Department of Clinical and Chemical pathology, Kasr Al-Ainy, Cairo University, Egypt.
[2]Department of Internal Medicine, Kasr Al-Ainy, Cairo University, Egypt.
*Corresponding author E-mail: mervat.naguib@kasralainy.edu.eg

Transcription factor 7-like 2 (*TCF7L2*) variants are known risk factors of type 2 diabetes (T2DM).However, this association is not consistent among different populations. The current study aimed at investigating the relationship between rs 7903146, rs 12255372 variants of *TCF7L2* and susceptibility to T2DM and different metabolic parameters in a cohort of Egyptian type 2 diabetic patients. This case control study included 60 diabetic patients and 60 matched unrelated healthy controls. Genotyping was performed by using Real Time-PCR. The frequency of genotypes, alleles, anthropometric measures, glycemic indices, HOMA-IR and lipid profile were evaluated in patients and control. Regarding rs 7903146, TT genotype was more frequent in healthy controls (43.3%) than diabetic patients (20%) (OR = 0.291, 95% CI = 0.108-0.788, *P* = 0.015). T allele was more frequent in healthy control (61.7%) than diabetic patients (44.2%) and it was associated with lower risk of diabetes (OR = 0.492, 95% CI = 0.294-0.823, *P* = 0.007). However, there was no significant difference between patients with CC, CT and TT genotypes of rs7903146 regarding HbA1C (p=0.549), HOMA-IR (p=0.359), total cholesterol (p=0.482) In contrast, T allele of rs12255372 had no significant relation to diabetes risk (OR = 0.602, 95% CI = 0.361-1.005, *P* = 0.052). There was no statistically significant difference of frequency of any rs12255372 genotypes between cases and controls. In addition, patients with GG,GT, TT genotypes of rs12255372 had no significant difference regarding HbA1C (p=0.393), HOMA-IR (p=0.985), total cholesterol (p=0.368). The study confirmed the association of *TCF7L2* (rs 7903146) and T2DM, while failed to detect any association between *TCF7L2* (rs 12255372) and susceptibility to T2DM. No significant difference in respect to metabolic parameters between different genotypes of rs7930146 and rs12255372.

**Keywords:** *TCF7L2*; genotype; type 2 diabetes.

Diabetes mellitus is a major public health problem worldwide and its prevalence has been increasing rapidly in developed and developing countries.[1] This prevalence is suspected to increase by 55% in 2035 to reach 591.9 million of adult's affected.[2] Type 2 diabetes mellitus (T2DM) represents the most common form of diabetes. The most likely explanation for the dramatic increase in T2DM prevalence observed over the past two decades is changing patterns of diet and physical activity. However, it has been assumed that these factors may cause T2DM only in the existence of genetic susceptibility.[3]

Genome-wide association studies (GWAS) have identified over 70 loci associated with T2DM including Transcription factor

7-like 2 (TCF7L2) gene.[4] TCF7L2 is located on chromosome 10q25.3.[5] TCF7L2 plays a master role in regulating insulin biosynthesis, secretion, and processing .[6] Moreover, TCF7L2 through the Wnt signaling pathway is essential for proliferation of the pancreatic epithelium and islet proliferation.[7] Recently, TCF7L2 was found to protect pancreatic cells against interleukin-1 and interferon induced cell apoptosis, stimulates cell proliferation and mediates glucose stimulated insulin secretion.[8]

rs7903146, rs12255372 variants are among the commonest single nucleotide polymorphisms (SNPs) of TCF7L2 that are connected to T2DM risk. Multiple studies among European, North Indian and African populations have confirmed the association of TCF7L2 variants to T2DM risk.[9-11] However, TCF7L2 variants had no association to T2DM in Euro-Brazilian individuals and in Arabic subjects .[12,13]

The present study was conducted to analyze the distribution of TCF7L2 rs7930146 and rs12255372 polymorphisms in a group of Egyptian patients with T2DM and healthy controls in an attempt to find an association that might exist between these two polymorphisms and the risk of developing T2DM and their effect on different laboratory parameters.

## MATERIALS AND METHODS

This case-control cross sectional study included 60 patients with T2DM (group 1) attending the Endocrinology and Diabetic Clinic at kasr al Ainy Hospitals, Cairo University and 60 normal unrelated subjects of the same age and sex taken as control (group 2) .The study protocol was approved by Institutional Ethical Committee of Kasr Al Ainy Hospital. All participants provided informed consent to participate in this study. The study protocol and procedures conform to the ethical guidelines of the 1975 declaration of Helsinki.

The patients were diagnosed based on to American Diabetes Association criteria for diabetes. Patients between 40-70 years and receiving oral hypoglycemic drugs were included in the study. Patients with type 1diabetes and diabetes secondary to endocrinopathies, pancreatic diseases or drugs have been excluded.

All patients and control subjects were subjected to thorough medical evaluation including determination of age, gender, blood pressure, anthropometric measures [weight, height and body mass index (BMI)]. Biochemical tests including: fasting blood glucose, fasting insulin, glycosylated hemoglobin (HbA1c), total cholesterol, high density lipoprotein cholesterol (HDL), Low density lipoprotein cholesterol (LDL), triglycerides. Insulin resistance status was determined using the HOMA-IR calculation, from the following equation: fasting insulin (microU/L) x fasting glucose (nmol/L)/22.5.[14] Detection of *TCF7L2* polymorphisms (rs 7930146, rs12255372) was done by real time PCR.

### Sample collection and biochemical assay

Eight ml of blood were collected from each patient and divided as follows: Two ml of plasma were withdrawn by aseptic venipuncture to a pre-chilled violet top EDTA vacutainer tubes for genomic DNA study, DNA samples were stored at -20°C to be used for TaqMan real time PCR; Two ml of blood were withdrawn to EDTA tube for measuring HbA1C;Two ml of blood were withdrawn to a red-topped serum separator tube, serum was harvested by centrifugation, samples were analyzed for serum insulin and lipid profile; and Two ml of blood was withdrawn into fluoride tube for measuring serum fasting glucose. Clinical chemistry analysis was done on Dimension RxL Max (Siemens,USA), while serum insulin was assayed on Cobas e 411(Rosh diagnostics, Germany).

### Isolation of Genomic DNA and SNP genotyping

DNA extraction and analysis was done using Gene JET Whole blood Genomic DNA Purification Mini Kit (Thermo Fisher, USA). The quantity of DNA was assessed on the basis of Qubit dsDNA BR assay kit 10 with the use of Qubit 2.0 fluorometer (Invitrogen, UK).

Genotyping of TCF7L2 polymorphisms (rs 7930146, rs12255372) was determined using RT-PCR allelic discrimination assays that were designed using Taq-Man SNP Genotyping Assays by using Step One Real-Time PCR systems (Applied Biosystems, Foster City, USA).The probes were labeled using the fluorescent dyes VIC and FAM. PCR reactions were run in a 25 UL final volume containing 12.5 UL of TaqMan Universal PCR Master Mix (2x), 1.25 UL of TaqMan SNP

Genotyping Assay (20x) and 20 ng of genomic DNA. Cycling conditions were 95°C for 10 min, 50 cycles of 92°C for 10 s and 60°C for 1 min. Controls were included in each run. Data analysis for allele discrimination was performed with the Applied Biosystems Real-Time PCR System software (Applied Biosystems, Foster City, USA).

**Statistical analysis**

Data were coded and entered using the statistical package SPSS version 23. Data was summarized using mean, standard deviation, median, minimum and maximum for quantitative variables and frequencies (number of cases) and relative frequencies (percentages) for categorical variables. Comparisons between groups were done using unpaired t test when comparing 2 groups and analysis of variance (ANOVA) with multiple comparisons post hoc test when comparing more than 2 groups. For comparing categorical data, Chi square ($\chi 2$) test was performed. Exact test was used instead when the expected frequency is less than 5. Genotype and allele frequencies were compared between the disease and the control groups using logistic regression. Odds ratio (OR) with 95% confidence intervals was calculated. P-values less than 0.05 were considered as statistically significant.

**Table 1.** Clinical and Laboratory data of the two studied groups

| Variable | Group (1)n=60 | Group (2)n=60 | *P value |
|---|---|---|---|
| [1]BMI(kg/m$^2$) | 31.89 ± 6.22 | 26.07 ± 2.66 | <0.001 |
| Waist circumference(cm) | 110.42 ± 10.77 | 101.28 ± 7.78 | <0.001 |
| Fasting glucose (mg/dl) | 168.72 ± 60.32 | 83.92 ± 7.99 | <0.001 |
| Fasting insulin (uU/mL) | 13.95 ± 16.83 | 5.35 ± 1.96 | <0.001 |
| [2]HOMA-IR | 5.6 ± 7.36 | 1.19 ± 0.49 | <0.001 |
| [3]HbA1$_C$ (%) | 8.46 ± 1.91 | 5.30 ± 0.39 | <0.001 |
| Triglygerides (mg/dL) | 178.02 ± 74.24 | 91.40 ± 31.34 | <0.001 |
| Total Cholesterol (mg/dL) | 210.58 ± 47.80 | 169.87 ± 27.36 | <0.001 |
| [4]HDL-C (mg/dL) | 37.83 ± 9.52 | 39.87 ± 10.10 | 0.259 |
| [5]LDL-C(mg/dL) | 136.48 ± 41.35 | 113.10 ± 27.65 | <0.001 |

Group 1: T2DM patients; Group 2: healthy controls. Values are mean ± SD. *P value <0.05 is considered significant.
[1]BMI: body mass index; [2]HOMA-IR Homeostatic model assessment of insulin resistance; [3]HbA1$_C$: glycated hemoglobin; [4]HDL-C: high density lipoprotein cholesterol;[5]LDL-C: low density lipoprotein cholesterol.

**Table 2.** Frequencies and univariate analysis of SNP genotypes and alleles in the two groups

| [1]SNP | Genotype/Allele | Group (1)n=60 | Group(2)n=60 | OR (95% CI) | *P value |
|---|---|---|---|---|---|
| rs7903146 | CC(n=31) | 19(31.7%) | 12(20%) | 1 | reference |
| | CT(n=51) | 29(48.3%) | 22(36.7%) | 0.833(0.335-2.070) | 0.693 |
| | TT(n=38) | 12(20%) | 26(43.3%) | 0.291(0.108-0.788) | 0.015 |
| | C allele | 67(55.8%) | 46(38.3%) | 1 | 0.007 |
| | T allele | 53(44.2%) | 74(61.7%) | 0.492(0.294-0.823) | |
| rs12255372 | GG(n=39) | 22(36.7%) | 17(28.3%) | 1 | reference |
| | GT(n=53) | 29(48.3%) | 24(40%) | 0.934(0.406-2.147) | 0.872 |
| | TT(n=28) | 9(15%) | 19(31.7%) | 0.366(0.133-1.010) | .052 |
| | G allele | 73(60.8%) | 58 (48.3%) | 1 | 0.052 |
| | T allele | 47(39.2%) | 62(51.7%) | 0.602(0.361-1.005) | |

Group 1: T2DM patients; Group 2: healthy controls.Values are n (%). *P value <0.05 is considered significant
[1]SNP: Single-nucleotide polymorphism

**RESULTS**

**Characteristics of studied groups**

The mean age of the studied patients was (55.83+10.9) years and 57.5% of them were female. The age and gender of participants didn't differ between patients and healthy control. Different clinical and laboratory characteristics of patients and healthy control are shown in table (1).

**rs7903146 genotypes and alleles in the two studied groups**

The frequency of rs7903146 genotypes in group 1 was (CT: 48.3%; CC: 31.7%; TT: 20%), while in group 2 was (CT: 36.7%; CC: 20%; TT:

43.3%). The TT genotype was associated with lower risk of diabetes (OR = 0.291, 95% CI = 0.108-0.788, P =0.015).

Upon examining the allelic discrimination, C allele was present in 55.8% of group 1 versus 38.3% of group 2, While T allele was found in 44.2% of group 1 versus 61.7% of group 2. T-allele distribution was found to be protective variant as its frequency was higher in healthy control (OR = 0.492, 95% CI =0.294-0.823, P = 0.007) (Table 2)

Clinical and laboratory data were compared according to the detected TCF7L2 rs7903146 genotypes (CC, CT, and TT) in group 1. Using one-way ANOVA, no statistically

**Table 3.** Comparison between TCF7L2 rs7930146 genotypes among diabetic patients as regards clinical and laboratory data

| Variable | CC(n=19) | CT(n=29) | TT(n=12) | *P value |
|---|---|---|---|---|
| [1]BMI(kg/m$^2$) | 34.53 ± 8.41 | 30.84 ± 4.30 | 30.25 ± 5.16 | 0.077 |
| Waist circumference(cm) | 113.63 ± 12.42 | 108.86 ± 10.30 | 109.08 ± 8.54 | 0.294 |
| Fasting glucose (mg/dl) | 154.42 ± 38.88 | 179.48 ± 75.16 | 165.33 ± 44.65 | 0.369 |
| Fasting insulin (uU/mL) | 16.19 ± 14.05 | 21.34 ± 19.19 | 18.84 ± 15.18 | 0.590 |
| [2]HOMA-IR | 6.10 ± 5.43 | 9.21 ± 8.16 | 8.38 ± 7.93 | 0.359 |
| [3]HbA1C (%) | 8.27 ± 1.83 | 8.37 ± 2.00 | 9.00 ± 1.84 | 0.549 |
| Triglygerides (mg/dL) | 176.05 ± 61.52 | 183.48 ± 88.31 | 167.92 ± 57.68 | 0.827 |
| Total Cholesterol (mg/dL) | 206.74 ± 39.36 | 217.86 ± 55.28 | 199.08 ± 40.37 | 0.482 |
| [4]HDL-C (mg/dL) | 36.84 ± 11.62 | 38.79 ± 9.02 | 37.08 ± 7.30 | 0.756 |
| [5]LDL-C (mg/dL) | 133.26 ± 38.48 | 141.93 ± 44.11 | 128.42 ± 40.37 | 0.592 |

Notes: Values are mean ± SD. *P value <0.05 is considered significant.
[1]BMI: body mass index; [2]HOMA-IR Homeostatic model assessment; [3]HbA1$_C$: glycated hemoglobin; [4]HDL-C: high density lipoprotein cholesterol;[5] LDL-C: low density lipoprotein cholesterol

**Table 4.** Comparison between TCF7L2 rs12255372 genotypes among diabetic patients as regards clinical and laboratory data

| Variable | GG(n=22) | GT(n=29) | TT(n=9) | *P value |
|---|---|---|---|---|
| [1]BMI(kg/m$^2$) | 33.27 ± 8.32 | 30.93 ± 4.65 | 31.62 ± 4.39 | 0.416 |
| Waist circumference(cm) | 111.36 ± 13.38 | 109.69 ± 9.20 | 110.44 ± 9.17 | 0.864 |
| Fasting glucose (mg/dl) | 172.27 ± 56.27 | 169.9 ± 70.1 | 156.22 ± 33.35 | 0.795 |
| Fasting insulin (uU/mL) | 18.34 ± 16.98 | 19.93 ± 17.08 | 19.03 ± 17.50 | 0.947 |
| [2]HOMA-IR | 7.85 ± 8.24 | 8.21 ± 6.39 | 8.09 ± 8.84 | 0.985 |
| [3]HbA1C (%) | 8.45 ± 1.81 | 8.23 ± 1.91 | 9.23 ± 2.11 | 0.393 |
| Triglygerides (mg/dL) | 163.45 ± 54.81 | 184.24 ± 88.80 | 193.56 ± 64.61 | 0.493 |
| Total Cholesterol (mg/dL) | 203.73 ± 34.12 | 219.52 ± 52.88 | 198.56 ± 58.52 | 0.368 |
| [4]HDL-C (mg/dL) | 38.09 ± 10.73 | 37.62 ± 8.27 | 37.89 ± 11.26 | 0.985 |
| [5]LDL-C (mg/dL) | 131.77 ± 35.34 | 138.72 ± 49.06 | 140.78 ± 28.43 | 0.797 |

Notes: Values are mean ± SD. *P value <0.05 is considered significant.
[1]BMI: body mass index; [2]HOMA-IR Homeostatic model assessment of insulin resistance; [3]HbA1$_C$: glycated hemoglobin; [4]HDL-C: high density lipoprotein cholesterol;[5] LDL-C: low density lipoprotein cholesterol

significant difference could be detected between the 3 genotypes in respect to waist circumference (p =0.294), HOMA-IR (p=0.359), HbA1c (p=0.549), LDL-cholesterol (p=0.592) and triglycerides (p=0.827) (Table 3).

**rs12255372 genotypes and alleles in the studied groups**

The detected genotypes of rs12255372 in group 1 were GG (36.7%), GT (48.3%) and TT (15%), while in group 2 were GG (28.3%), GT (40%) and TT (31.7%).The frequencies of different genotypes was not significantly differ between both groups. (Table 2).

The frequency G allele was not significantly different between group 1 (60.8%) and group 2 (48.3%).also; T allele frequency didn't differ between both groups and it was not associated with increased diabetes risk (OR = 0.602, 95% CI = 0.361-1.005, *P* = 0.052).

When we classified the diabetic patients according to their rs12255372 genotypes (GG, GT, TT), there was no significantly difference between the 3 genotypes regarding any of the clinical or laboratory parameters (Table 4).

**DISCUSSION**

Diabetes is a growing heath problem of multifactorial origin, understanding its genetic background might help in early detection and disease prevention. *TCF7L2* genetic variants have been closely linked to T2DM risk, yet this association in the Egyptian population needs further evaluation.[9-11] The aim of the current study was to investigate the relationship between *TCF7L2* polymorphisms rs 7903146, rs 12255372 and susceptibility to Type 2 diabetes mellitus in a group of Egyptian population and healthy control.

Based on our findings, T allele of *TCF7L2* rs7903146 was associated with lower risk of T2DM, and TT genotype frequency was higher in healthy subjects. Different studies yielded controversial results in different ethnic groups. In agreement with the current work; the T allele was protective against diabetes in North Indian population and Cameroon population.[10,11] conversely, in Sudanese and a Turkish population the T allele at rs7903146 was strongly associated with T2DM risk.[15,16] In addition, there was no

association between rs 7903146 variant and T2DM in Euro-Brazilian individuals.[17]

A recent meta- analysis by Guan *et al*. revealed that the rs7903146 T allele of the TCF7L2 gene was positively linked with higher T2DM susceptibility.[18] Same results were obtained by another meta-analysis involving 10 studies related to the rs7903146 loci and risk of T2DM; this included 3404 cases of T2DM patients and 6473 control cases.[19]

Also, the frequency of TT genotype in our study was significantly lower in diabetic patients compared to healthy control. Verma *et al*. reported the same finding in subjects from North India and same and results obtained in Aferican population.[10,11]

As regards *TCF7L2* (rs 12255372), in agreement with the current work, a Venezuelan study found that the genotypic and allelic frequencies for the *TCF7L2* rs12255372 polymorphism did not differ significantly between the diabetic patients and healthy subjects.[20] In contrast to our results, T allele of rs12255372 was found to be a risk factor for diabetes in a Cameroonian population and a Chines population.[21,22] While, rs12255372 G allele was the risk allele in Chinese Hui population.[23]

A meta-analysis of 40 studies showed an effect of rs12255372 on type 2 diabetes risk (OR: 1.33, 95% CI: 1.27–1.40), Whereas in some ethnic populations including African, American, Pima Indian, Mexican American and Arab no significant association was found.[24]

Several studies investigated both SNPs (rs7930146, rs12255372) together in relation to T2DM. A study by Barros *et al*., in northern Brazil showed that the genotype and allele frequencies of rs7903146 and rs12255372 were not significantly associated with T2DM risk.[25] In contrast, a study by Shokouhi *et al*. done in a sample of the Kurdish population of Iran found that the T-allele of rs12255372 and rs7903146 polymorphisms of *TCF7L2* was risk allele for T2DM.[26]

To date, no major GWAS for diabetes has been reported for an Arab population. Replication studies conducted in Arabs have given mixed results.[27] For example, a study of Saudi Arabian and a study for United Arab Emirates population reported weak or no association of rs7903146 and rs12255372 with T2DM, while a study of

Arab Tunisian showed association of T- allele of rs7903146 and rs12255372 with T2DM.[13,28,29]

Likewise our results, a Brazilian study showed that the T allele was significantly associated with T2DM risk, while no significant difference was found among the three rs7903146 SNP genotypes regarding blood pressure, BMI, lipid profile, and HbA1c.[30] The association of *TCF7L2* with T2DM independently of BMI, insulin resistance and other metabolic indices confirms that impaired insulin secretion is a result of genetic defect *of TCF7L2*.[31]

Similar to our findings, In T2DM Iranian population, no significant difference was found in any of the clinical or biochemical parameters between GG and GT + TT genotypes of rs12255372.[26]

In contrast to our study, CT + TT genotypes were significantly associated with the lower levels of total cholesterol the CC genotype of rs7903146 in Iranian Kurdish ethnic population.[26] Also, in a group of Italian elderly population, the TCF7L2 rs7903146 polymorphism was associated with lower insulin levels, and lower risk lipid profiles.[32] The reason for these discrepancies between the studies, including our study might be related to different factors such as the study design, sample size, population heterogeneity, and environmental factors.[33]

## CONCLUSIONS

*TCF7L2* was related to T2DM in the studied Egyptian population and both T allele and TT genotype of rs7903146 were associated with lower risk of T2DM. However, no significant association was found to any of rs12255372 genotypes or alleles. Moreover, there was no significant difference in any of metabolic parameters between different genotypes of rs7930146 and rs12255372.

## ACKNOWLEDGEMENT

## REFERENCES

1.  Syamsurizal S, Yanwirasti Y, Manaf A, *et al*. Transcription factor 7-like 2 as type-2 diabetes mellitus diagnostic marker in ethnic Minangkabau. *Universa Medicina*; **33**: 205-212 (2015).
2.  International Diabetes Federation. IDF Diabetes Atlas. 6th ed. Brussels (2013).
3.  De Souza BM, Brondani LA, Bouças AP, *et al*. Associations between UCP1-3826A/G, UCP2-866G/A, Ala55Val and Ins/Del, and UCP3-55C/T polymorphisms and susceptibility to type 2 diabetes mellitus: case-control study and meta-analysis. *PloS one*; **8**: e54259 (2013).
4.  Hara K, Shojima N, Hosoe J, *et al*. Genetic architecture of type 2 diabetes. Biochemical and *biophysical research communications*; **452**: 213-220 (2014).
5.  Duval A, Busson-Leconiat M, Berger R, *et al*. Assignment of the TCF-4 gene (TCF7L2) to human chromosome band 10q25.3.*Cytogenet Cell Genet*; **88**:264-5 (2000).
6.  Basile KJ, Guy VC, Schwartz S, *et al*. Overlap of genetic susceptibility to type 1 diabetes, type 2 diabetes, and latent autoimmune diabetes in adults. *Curr Diab Rep*; **14**:550 (2014).
7.  Cauchi S, Meyre D, Dina C, *et al*. Transcription factor TCF7L2 genetic study in the French population: expression in human beta-cells and adipose tissue and strong association with type 2 diabetes. *Diabetes* 2006; **55**: 2903–2908.
8.  Jin T. Current understanding on role of the Wnt signaling pathway effector TCF7L2 in glucose homeostasis. *Endocrine reviews*; **37**: 254-277 (2016).
9.  Florez JC, Jablonski KA, Bayley N, et al. TCF7L2 polymorphisms and progression to diabetes in the Diabetes Prevention Program. *N Engl J Med*; **355**:241 –250 (2006).
10. Verma S, Srivastava N, Banerjee M. PS 04-25 Association of tcf7l2 and ppar [gamma] gene variants with type 2 diabetes mellitus in north indian population .*Journal of Hypertension*; **34**: e139-e140 (2016).
11. Guewo-Fokeng M, Sobngwi E, Atogho-Tiedeu B, *et al*. Contribution of the TCF7L2 rs7903146 (C/T) gene polymorphism to the susceptibility to type 2 diabetes mellitus in Cameroon .*Journal of Diabetes & Metabolic Disorders*; **14**: 1 (2015).
12. De Melo SF, Frigeri HR, dos Santos-Weiss IC, *et al*. Polymorphisms in FTO and TCF7L2 genes of Euro-Brazilian women with gestational diabetes.

*Clin Biochem*; **48**: 1064-7 (2015).

13. Alsmadi O, Al-Rubeaan K, Mohamed G, *et al* . Weak or no association of TCF7L2 variants with Type 2 diabetes risk in an Arab population. *BMC Med Genet*; **9**: 72 (2008).

14. Salgado AL, Carvalho LD, Oliveira AC, *et al*. Insulin resistance index (HOMA-IR) in the differentiation of patients with non-alcoholic fatty liver disease and healthy individuals. *Arquivos de gastroenterologia*; **47**: 165-169 (2010).

15. Ibrahim AT, Hussain A, Salih MA, *et al*. Candidate gene analysis supports a role for polymorphisms at TCF7L2 as risk factors for type 2 diabetes in Sudan. *Journal of Diabetes & Metabolic Disorders*; **15**:4 (2016).

16. Demirsoy IH, Aras N, Cinkir U. TCF7L2 rs7903146 Gene Variation Is Associated with Risk of Type 2 Diabetes in Turkish Population. *J Clin Med Genom*; **4**: 2 (2016).

17. Welter M, Frigeri HR, Souza EM, *et al*. Polymorphisms in the Fat Mass and Obesity Associated ( FTO ) and Transcription factor 7-like 2 ( TCF7L2 ) genes in Euro-Brazilian individuals with type 2 diabetes. *Diab obes Metab Diso OA* ; **2**:1-6 (2016).

18. Guan Y, Yan LH, Liu XY, *et al*. Correlation of the TCF7L2 (rs7903146) polymorphism with an enhanced risk of type 2 diabetes mellitus/ : a meta-analysis. *Genet Mol Res* ; **15**: 3 (2016).

19. Liu XH, Xie CG, An Y, *et al*. Meta-analysis of the association between the rs7903146 polymorphism at the TCF7L2 locus and type 2 diabetes mellitus susceptibility. *Genet Mol Res*; **14**:16856-62 (2015).

20. Moran Y, Labrador L, Camargo ME, *et al*. Design of an allele-specific PCR assay to genotype the rs12255372 SNP in a pilot study of association between common TCF7L2 polymorphisms and type 2 diabetes in Venezuelans. *Archives of endocrinology and metabolism*; **60**: 246-251 (2016).

21. Nanfa D, Sobngwi E, Atogho-Tiedeu B, *et al*. Association between the TCF7L2 rs12255372 (G/T) gene polymorphism and type 2 diabetes mellitus in a Cameroonian population: a pilot study. *Clinical and translational medicine*; **4**: 17 (2015).

22. Yao H, Wang Z, Wang T, *et al*. Association of TCF7L2 genetic polymorphisms with type 2 diabetes mellitus in the Uygur Population of China. *International journal of environmental research and public health*; **12**: 11797-11814 (2015).

23. Yang Y, Xu JR, Wang YJ, *et al*. Association of TCF7L2 gene polymorphisms with susceptibility to type 2 diabetes mellitus in a Chinese Hui population. *Genet Mol Res*; **14**:10064-71 (2015).

24. Peng S, Zhu Y, Lü B, *et al*. TCF7L2 gene polymorphisms and type 2 diabetes risk: a comprehensive and updated meta-analysis involving 121174 subjects. *Mutagenesis*; **28**: 25-37 (2013).

25. Barros CM, Araujo-Neto AP, Lopes TR, *et al* . Association of the rs7903146 and rs12255372 polymorphisms in the TCF7L2 gene with type 2 diabetes in a population from northeastern Brazil. *Genet Mol Res*; **13**: 7889-98 (2014).

26. Shokouhi S, Delpisheh A, Haghani K, *et al*. Association of rs7903146, rs12255372, and rs290487 polymorphisms in TCF7L2 gene with type 2 diabetes in an Iranian Kurdish ethnic group. *Clin Lab*; **60**:1269–1276 (2014).

27. Siddiqui K, Tyagi S .Genetics, genomics and personalized medicine in Type 2 diabetes: a perspective on the Arab region. *Personalized Medicine*; **12**: 417-431 (2015).

28. Saadi H, Nagelkerke N, Carruthers SG, *et al*. Association of TCF7L2 polymorphism with diabetes mellitus, metabolic syndrome, and markers of beta cell function and insulin resistance in a population-based sample of Emirati subjects. *Diabetes research and clinical practice*;    : 392-398 (2008).

29. Turki A, Al-Zaben GS, Mtiraoui N, *et al*. Transcription factor-7-like 2 gene variants are strongly associated with type 2 diabetes in Tunisian Arab subjects. *Gene* ; **513**: 244-248 (2013).

30. Assmann TS, Duarte GC, Rheinheimer J, *et al*.The TCF7L2 rs7903146 (C/T) polymorphism is associated with risk to type 2 diabetes mellitus in Southern-Brazil. Arquivos Brasileiros de *Endocrinologia & Metabologia*; **58**: 918-925 (2014).

31. Tong Y, Lin Y, Zhang Y, *et al*. Association between TCFL2 gene polymorphisms and susceptibility to type 2 diabetes mellitus: a large Human Genome Epidemiology (HuGE) review and meta-analysis. *BMC Med Genet*; **10**:15 (2009).

32. Melzer D, Murray A, Hurst AJ, *et al*. Effects of the diabetes linked TCF7L2 polymorphism in a representative older population. *BMC Med* ; **4** :34 (2006).

33. Haghani K, Bakhtiyari S. The study on the relationship between IRS-1 Gly972Arg and IRS-2 Gly1057Asp polymorphisms and type 2 diabetes in the Kurdish ethnic group in West Iran. *Genet Test Mol Biomarkers*; **16**:1270-6 (2012)