http://www.pjbs.org

PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

OPEN ACCESS Pakistan Journal of Biological Sciences

ISSN 1028-8880
DOI: 10.3923/pjbs.2022.218.225

Research Article
Possible Role of rs7903146 Polymorphism of the Transcription Factor 7-Like 2 Gene in Genetic Predisposition to Type 2 Diabetes

Lala A. Akhundova, Zarintaj R. Rustamova, Gulmira R. Alibayova, Nurmammad Sh. Mustafayev and Irada M. Huseynova

Department of Fundamental Problems of Biological Productivity, Laboratory of Population Genomics, Institute of Molecular Biology and Biotechnologies, Baku, Azerbaijan

Abstract

Background and Objective: The association of rs7903146 polymorphism of TCF7L2 gene with Type 2 diabetes mellitus found almost in all ethnic groups. Therefore, the current study focused on estimating this association in the Azerbaijan population for the 1st time. **Materials and Methods:** A study was conducted on 110 patients with Type 2 diabetes mellitus and 115 healthy controls. The biochemical parameters were analyzed and calculated with an independent t-test and Fisher exact test. DNA extracted from the blood samples run in PCR. PCR was used to detect the presence of TCF7L2 rs7903146 C/T polymorphism and the products of the PCR were visualized in a 1.5% gel electrophoresis. **Results:** According to the obtained data, T allele and TT genotype of rs7903146 polymorphism strongly correlated with the risk for Type 2 diabetes (odds ratio of 1.68 for T allele and $p = 0.007$, odds ratio of 3.9 for TT genotype, $p = 0.0028$). These results were adjusted by applying the recessive model ($p = 0.003$). Moreover, the biochemical parameters also show a significant difference in the fasting glucose level ($p = 0.0001$), fasting insulin ($p = 0.0001$), BMI ($p = 0.0002$) and age ($p = 0.015$) inpatient and control groups. **Conclusion:** Based on the results, the TCF7L2 rs7903146 (C>T) polymorphism is the genetic risk factor related to Type 2 diabetes in the Azerbaijan population.

Key words: Diabetes mellitus, gene, single nucleotide polymorphism, fasting glucose level, rs7903146 polymorphism

Citation: Akhundova, L.A., Z.R. Rustamova, G.R. Alibayova, N.Sh. Mustafayev and I.M. Huseynova, 2022. Possible role of rs7903146 polymorphism of the transcription factor 7-like 2 gene in genetic predisposition to type 2 diabetes. Pak. J. Biol. Sci., 25: 218-225.

Corresponding Author: Nurmammad Sh. Mustafayev, Department of Fundamental Problems of Biological Productivity, Laboratory of Population Genomics, Institute of Molecular Biology and Biotechnologies, Baku, Azerbaijan

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Diabetes Mellitus (DM) is a chronic metabolic disease caused by metabolic disorders due to a deficiency in insulin secretion and an increase in blood sugar. DM is an acute social and medical problem. About 5% of the global population suffers from DM and the number of patients doubles every 15 years. Type 2 Diabetes (T2D) is the most common type of diabetes that characterized by several processes like dysfunction of β-cells with a decrease in insulin secretion, increased secretion of glucagon, decreased incretin response, a decrease in β-cell mass, increased glucose production in the liver, increased glucose reabsorption, activation of lipolysis processes, decreased glucose uptake by muscles and neurotransmitter dysfunction[1]. For today, >100 genes are associated with T2D. The most prominent genes that show strong association with T2D are Transcription Factor 7-Like 2 (TCF7L2), Potassium Inwardly Rectifying Channel Subfamily J Member 11 (KCNJ11), Peroxisome Proliferator Activated Receptor Gamma (PPARG), ATP Binding Cassette Subfamily C Member 8 (ABCC8), Calpain 10 (CAPN10), potassium Voltage-Gated Channel Subfamily Q Member 1 (KCNQ1), Hematopoietically Expressed Homeobox (HHEX), Adiponectin (ADIPOQ).

Microsatellite of TCF7L2 gene found in intron 4 and investigated in the Icelandic population was 1st identified as a risk factor of diabetes in 2006[2]. Afterwards, this microsatellite was found to be highly associated with one of the TCF7L2 gene polymorphisms, rs7903146 (also located in intron 4). TCF7L2 gene encoding the protein that is a transcription factor and is a member of the Wnt/β-catenin signalling system. TCF7L2 gene consists of 19 exons (five of which are alternative) and is located on chromosome 10 in the region of 10q25.2-q25.3. High expression of TCF7L2 was detected in different tissues like kidney, brain, lung, liver adipose tissues and pancreatic β-cells[3]. The exact mechanisms by which different Single Nucleotide Polymorphisms (SNPs) of the TCF7L2 gene can reduce insulin secretion are uncertain. Nowadays, 2 versions are known: By direct influence on the pancreatic β-cells or via the WNT pathway[4,5]. TCF7L2 regulate the expression of the proglucagon gene (GCG) that is located in intestinal L-cells, by the WNT signalling pathway. The GCG regulate the expression of incretin hormones like Glucagon-Like Peptide-1 (GLP-1)[6]. Meanwhile, the GLP-1 maintain glucose homeostasis by increasing insulin secretion and simultaneously inhibit glucagon secretion as well as playing an important role in the differentiation and proliferation of pancreatic islet cells. Therefore, polymorphisms of the TCF7L2 gene may reduce the incretin response to oral nutrients and consequently reduce insulin secretion. Moreover, Lyssenko *et al.*[7] showed that the TCF7L2 do not influence the level of another peptide that is part of the incretin family of hormones, Glucose-dependent Insulinotropic Peptide (GIP). GIP stimulate the secretion of glucagon and insulin while inhibiting gastric acid secretion. Interesting that the study of Holst *et al.*[8] also showed that while the level of GLP-1 in T2D patients is impaired, the level of GIP is near to normal. Patients with T2D showed overexpression of TCF7L2 at the mRNA level in pancreatic islets in comparison with non-diabetic individuals. Recent studies also showed a strong positive association between the TCF7L2 gene and colorectal cancer[9]. It is known that b-catenin/T-Cell Factor (TCF) mediates regulation of Wnt/β-catenin pathway through transcriptional activation of Wnt-target genes like Adenomatous Polyposis Coli (APC) gene. The Wnt/β-catenin pathway is strongly associated with cancer development and progression[10]. Moreover, a high concentration of Insulin and Insulin-like Growth Factors (IGF) during diabetes might also stimulate cancer progression[11]. In addition, TCF7L2 was found to be important in the formation of the intestinal epithelium[12].

Two main SNPs (rs12255372 (G/T) and rs7903146 (C/T)) of TCF7L2 gene have a strong correlation with the risk of development of T2D and the presence of mutant T allele of both SNP is affected on glucagon inhibition as well as insulin secretion[7,13,14]. A study by Lyssenko *et al.*[7] showed that despite the concentration of fasting glucagon and GIP were normal in T2D patients, carriers of the T allele showed a strong positive correlation between GIP and glucagon while no significant correlation was observed among carriers of the wild C allele. In addition, patients with risk TT genotype are characterized by an increased rate of Endogenous Glucose Production (EGP), which is probably because of the glucose generation by the liver. Moreover, carriers of CT and TT genotypes showed a reverse correlation of TCF7L2 mRNA and insulin secretion after glucose stimulation. These data suggest that TCF7L2 gene expression influences insulin expression, especially on post-transcriptional level[7]. Folsom *et al.*[9] showed that homozygous for the TT genotype T2D patients have a higher risk of colon cancer than those who are homozygous for the CC genotype. The mechanism that maintains the association between rs7903146 and colon cancer is under investigation. Probably, another mutation is in linkage disequilibrium with rs7903146 that affect gene function. However, the carcinogenic effect might occur due to the Wnt/β-catenin signalling pathway. Normally β-catenin interacts with TCF7L2 to activate gene expression. Thus, mutations in genes like TCF7L2 that modulate β-catenin might promote activation or inhibition of genes that regulate cancer progression like the

APC gene[15]. The risk allele of rs7903146 showed has a high correlation with the risk of T2D in different ethnicities like white Europeans, West Africans, Mexican Americans, Indians and Japanese populations. Moreover, the associations of this polymorphism with T2D are found in populations that are ethnically close to Azerbaijan like Turkey, Iran, Iraq, Pakistan and Kazakhstan[16-20]. This study aimed to investigate the role of rs7903146 polymorphism of the TCF7L2 gene as a risk factor for T2D in the Azerbaijan population.

MATERIALS AND METHODS

Study area: This study was conducted from February to May of 2021 in the Institute of Molecular Biology and Biotechnologies. The blood samples were collected from the hospital of the Sumgait city, Azerbaijan.

Population studied: The fresh blood samples were collected voluntarily from 225 individuals of different ages and with different professional activities. The studied population sample includes the following groups: 115 controls and 110 patients. The biochemical characteristics like age, gender, fasting blood glucose, fasting insulin, BMI, total cholesterol, triglyceride, High Density Lipoprotein (HDL)-cholesterol and Low Density Lipoprotein (LDL)-cholesterol of each individual collected from the medical reports of diabetic patients and controls.

DNA isolation procedures: DNA from 200 µL blood samples was isolated using the "Diatom™ DNA Prep 200" kit (Izogen, Russian Federation) on manufacturer protocols. The concentrations and purity of the DNA samples were determined Spectrophotometrically in Epoch™ Microplate Spectrophotometer (BioTek, Agilent, USA) using Gene5 software. DNA samples were diluted individually before PCR. DNA samples were stored at -80°C.

rs7903146 genotyping: TCF7L2 gene rs7903146 polymorphism on 4th intron determined by Polymerase Chain Reaction (PCR) using 2 forward and one common reverse specific primers in Table 1[21].

A final reaction volume of 25 µL for the PCR contained 3 µL of genomic DNA, 1 µL of each primer, 1.5 µL of $MgCl_2$, 0.5 µL of Deoxynucleotide Triphosphate (dNTP), 0.2 µL of Taq DNA polymerase (PROMEGA), 2.5 µL of buffer and 15.2 µL of nuclease-free water. PCR conditions were as follows: Initial denaturation for 3 min at 95°C, followed by 35 cycles of 20 sec at 95°C, 30 sec at 60°C and 30 sec at 72°C and final extension for 5 min at 72°C. The obtained DNA fragments were separated by electrophoresis on a 1.5% agarose gel followed by staining with ethidium bromide and visualized under a UV transilluminator. The sizes of fragments were estimated by comparison with previously known molecular weight markers M100. The polymorphism detected by PCR was evident as a 205 bp fragment in the presence of the C or T allele.

Ethical considerations: This study was approved by the Ethics Committee of the Institute of Molecular Biology and Biotechnology, Azerbaijan National Academy of Science. All procedures are done following the international ethics rules as well as the ethical guidelines prepared by the Council for International Organizations of Medical Sciences[22]. All patients provided their informed consent to participate in this study. Blood samples as well as isolated from the blood DNA used only for research purposes.

Statistics: Biochemical characteristics calculated with one-tailed t-test and Fisher exact test and the data expressed as Means±Standard deviations. Hardy-Weinberg equilibrium and association between genotype and allele distribution in diabetic and control groups calculated through chi-square test with 2×2 contingency table. In addition, the chi-square test was used to determine the best genetic model that shows the association between rs7903146 SNP at the TCF7L2 locus and T2D. Odds Ratio (OR) and 95% confidence interval (95%) were calculated via an online available statistic calculator (http://vassarstats.net/odds2x2.html) to determine diabetes risk for the genotypes. Values of *p<0.05, **p<0.01 and ***p<0.001 considered as significant.

Table 1: List of used primer pairs and their related sequences

Target SNP	Primers	
rs7903146 (C/T)	F1 5'-GAACAATTAGAGAGCTAAGCACTTTTTAGAAAC-3'	For allele C detection
	F2 5'-GAACAATTAGAGAGCTAAGCACTTTTTAGAGAT-3'	For allele T detection
	CR 5'-AGATGAAATGTAGCAGTGAAGTGC-3	Common reverse primer

RESULTS

Characteristics of the studied population: For the research, 115 control and 110 T2D patients were selected. The group of patients consist of 75 males and 35 females. The biochemical characteristics are given in Table 2.

The significant differences between the 2 groups observed for age (63.45 vs. 59.77, p = 0.015), Body Mass Index (BMI) (28.6 vs. 25.4, p = 0.0002), fasting blood glucose concentration (11.2 vs. 4.39, p = 0.0001) and fasting insulin (17.6 vs. 7.3, p =0.0001). However, for the gender, Low Density Lipoprotein (LDL-cholesterol), total cholesterol, High Density Lipoprotein (HDL-cholesterol) and triglyceride no significant association observed between the 2 groups (p>0.05).

Association of the TCF7L2 rs7903146 polymorphism with the development of Type 2 diabetes mellitus: Two hundred twenty-five cases were positive for genotyping and represented on agarose gel by presence or absence of band of 205 bp in Fig. 1.

The presence of the C allele or CC genotype characterized the wild type while the T allele or TT genotype indicates the presence of the mutation. Genotype and allele distribution for rs7903146 polymorphism in diabetic patients as well as in controls is presented in Table 3.

The studied allele and genotype frequencies were in the Hardy-Weinberg equilibrium in both T2D patients and control groups. The frequency of CC, CT and TT genotype were, respectively 28, 50 and 22% in the case group and 42, 46 and 12% in the control group. The TT genotype vs CC genotype in patients group correlate with high risk for T2D (OR = 3.9, 95%CI = 1.59-9.57, p = 0.0028). The frequency of C and T alleles were, respectively 53 and 47% in the patient's group and 65 and 35% in the control group. In addition, homozygous carriers for the T allele (p = 0.0028) were identified with increased risk of diabetes in comparison with heterozygous carriers CT which show no association with T2D (p = 0.3482). In addition, the T allele was more frequent in the T2D patient group compared to the healthy control. The T allele vs C allele is correlated with an increased risk of T2D (OD = 1.68, 95%CI = 1.15-2.45, p = 0.007). In addition, relative risk calculated for carriers of homozygous T allele indicate that this allele positively correlated with the risk for disease development (RR = 1.3). Moreover, the result of relative risk among TT carriers showed a 1.6 times higher

Table 2: Clinical data of the study population

Variables	T2D (n = 110)	Control (n = 115)	p-value
Age (years)	63.45±8.9	59.77±11.9	0.015*
Sex			
Male	75	67	0.13
Female	35	48	
BMI (kg m^{-2})	28.6±2.7	25.4±2.4	0.0002***
Fasting blood glucose (mmol L^{-1})	11.24±3.7	4.39±0.68	0.0001***
Fasting insulin (μU mL^{-1})	17.6±7.2	7.3±2.9	0.0001***
Total cholesterol (mmol L^{-1})	8.09±2.87	7.19±2.27	0.14
Triglyceride (mmol L^{-1})	5.28±3.6	3.8±2.37	0.07
HDL-cholesterol (mmol L^{-1})	1.9±0.87	2.1±1.01	0.31
LDL-cholesterol (mmol L^{-1})	4.9±1.9	4.4±1.47	0.155

Data expressed as Means±SD, T2D: Type 2 diabetes mellitus, BMI: Body mass index, HDL-cholesterol: High-density lipoprotein cholesterol, LDL-cholesterol: Low-density lipoprotein cholesterol, p-values refer to independent t-test and Fisher's exact test, *p<0.05, **p<0.01 and ***p<0.001

Table 3: Genotype and allele frequency of the TCF7L2 rs7903146 (C/T) gene polymorphism in patients with T2D and controls

Genotypes	T2D (n = 110)	Control (n =115)	OR	95% CI	Chi-square	RR	p-value
CC	30 (28%)	44 (42%)	-	-	-	-	-
CT	56 (50%)	62 (46%)	1.32	0.73-2.38	0.88	1.1	0.3482
TT	24 (22%)	9 (12%)	3.9	1.59-9.57	9.46	1.8	0.0028**
Alleles							
C	116 (53%)	150 (65%)	-	-	-	-	-
T	104 (47%)	80 (35%)	1.68	1.15-2.45	7.26	1.3	0.007**
Genetic models							
Dominant (TT+CT) vs. CC	-	-	1.65	0.94-2.90	3.08	1.3	0.08
Recessive TT vs. (CT+CC)	-	-	3.28	1.45-7.44	8.79	1.6	0.003**
Co-dominant CC vs. (CC+TT)	-	-	0.88	0.52-1.49	0.2	0.94	0.65

T2D: Type 2 diabetes mellitus, OR: Odds ratio, CI: Confidence intervals, RR: Relative risk, RR>1, the association is positive, p-values: *p<0.05*, **p<0.01 and ***p<0.001 refer as significant

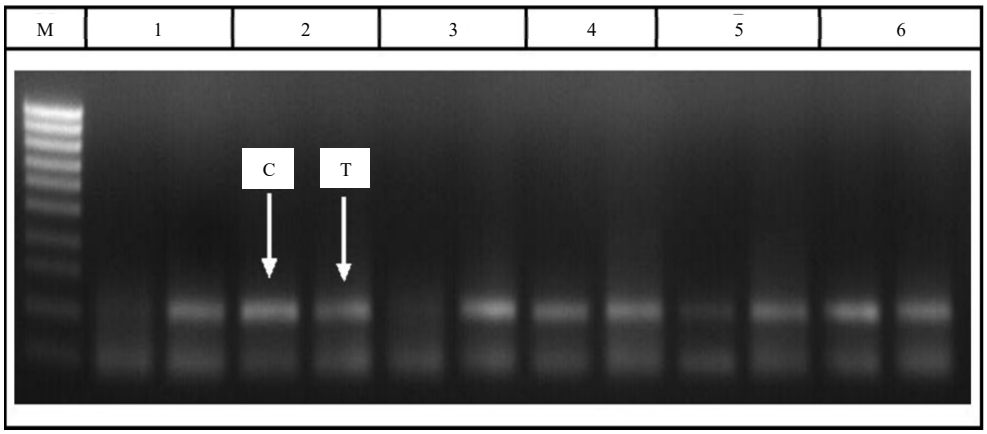

Fig. 1: TCF7L2 rs7903146 (C/T) gene polymorphism detection by polymerase chain reaction analysis

PCR products were visualized in 1, 5% agarose gel followed by staining with ethidium bromide and exposure to UV rays. The expected product size was 205 bp and mutation was detected by the presence of the mutant T allele. Lane M indicates a 100 bp DNA ladder, lane 2 indicates the presence of allele C and allele T (CT genotype) at 205 bp in the genotype of the patient with T2D

association with T2D in comparison to carriers of CT genotype. In addition, positive association between the T2D risk and the presence of rs7903146 (C/T) SNP of TCF7L2 gene polymorphism observed under dominant, recessive and co-dominant genetic models: Dominant model (TT+CT versus CC or = 1.65, 95% CI = 0.94-2.90, p = 0.08), recessive model (TT versus CT+CC: OR = 3.28, 95% CI = 1.45-7.44, p = 0.003), co-dominant model (CC versus CC+TT or = 0.88, 95% CI = 0.52-1.49, p = 0.65). Thus, the recessive model with an odds ratio of 3.28 fits the best to show the correlation between TCF7L2rs7903146 (C/T) polymorphism and the T2D susceptibility. Meanwhile, no association was observed in the dominant and co-dominant models.

DISCUSSION

DM is a global problem that has grown over the years. According to the International Diabetes Federation, diabetes affects 371 million people in the world, which is 7% of the total population of the Earth. There are different environments as well as genetic factors that contribute to diabetes formation. Polymorphisms in different genes increase the risk for diabetes. One of the polymorphism is the rs7903146 (C/T) polymorphism of the TCF7L2 gene. This SNP is frequently observed as a T2D-related variant. By using Mantel-Haenszel statistical test, the relative risk of rs7903146 in the formation of T2DM was found to be 2.41[2].

Polymorphisms of the TCF7L2 gene were detected in almost all ethnics groups except several Arab countries[23,24]. It was also studied and positively correlated with the risk of T2D in several populations like Iran, Pakistan, Turkey, Kazakhstan and Iraq, which are ethnically closed to Azerbaijan[16-20,25]. Recent meta-analysis including 34232 cases and 22396 controls concerning various ethnic groups of Caucasia, East and South Asian population revealed a significant positive association ($p<0.0001$) between rs7903246 (C/T) SNP polymorphism and risk of development of the T2D[26]. Besides the fact that this polymorphism was studied in different populations, it was never studied in the Azerbaijan population before.

Therefore, this study directed to find the correlation between polymorphism of rs7903146 of the TCF7L2 gene and susceptibility to T2D in the Azerbaijan population. The biochemical analyses show a high fasting blood glucose level in the patient's group (11.24 mmol L^{-1} or 202 mg dL^{-1}) compare to the control group (4.39 mmol L^{-1} or 79 mg dL^{-1}). Usually, around 40% of diabetic patients who had fasting glucose levels above 120 mg dL^{-1} suffer from postprandial hyperglycemia[27]. Correlation between the TT genotype of rs7903146 polymorphism and high fasting glucose levels observed among Caucasian adults ($p = 0.0389$) population but not in African Americans ($p = 0.2826$)[28]. In the current study, diabetic patients who showed a fasting glucose level higher than 180 mg dL^{-1} were predominantly carriers of CT genotype. Thus, it might assume that rs7903146 affects glucose metabolism. The association of the TCF7L2 rs7903146 polymorphism and the risk of T2D assed by PCR and visualized by using gel electrophoresis. The frequency of the T allele was found to be 47% in the diabetic group and 35% in the control group. This result was similar with those found in the population of Tatars (41.7% (T2D) and 30.8% (Control)), among Kurdish population of Iraq (40.6% (T2D) and 29.2% (Control)) and Khyber Pakhtunkhwa population of Pakistan (47% (T2D) and 35% (Control))[17,29,30]. However, the data do not

correlate with previously reported studies found in the Iranian (36% (T2D) and 29% (Control)) and Brazilian populations (28% (T2D) and 29% (Control))[31,32]. Differences may occur due to the sample size, ethnic background or influence of different environmental factors like diet or lifestyle. The genotypic distribution of rs7903146 (C/T) polymorphism was as follows: CC genotype found in 28% of patients and 42% of controls, CT genotype found in 50% of patients and 46% of controls and TT genotype found in 22% of patients and 12% of the control group. Thus the frequency of TT genotype was significantly higher in the diabetic group in comparison to the control group with OR 3.9 (95% CI =1.59-9.57) and p = 0.0028. Moreover, the T allele was found to be associated with a high risk of T2D development in the Azerbaijan population with an OR 1.68 (95% CI = 1.15-2.45, p = 0.007). In comparison to the carriers of TT genotype carriers of CT genotype were found to be under lower risk of T2D development (OR = 1.32 (95%CI = 0.73-2.38), p = 0.3482). In addition, the frequency of the CC genotype was 1.5 times higher in the control group, which prove the mutagenic role of the T allele in the development of T2D. These findings are consistent with the statement that carriers of CT and TT genotype have 1.4 and 2 times greater risk for T2D development than carriers of CC genotype in Caucasian, Hispanic-American, Japanese and African-American populations[33]. In addition, the association between the rs7903146 (C/T) polymorphism of the TCF7L2 gene and T2D was estimated by performing dominant, recessive and co-dominant genetic models. The analysis performed in the recessive genetic model showed the strongest association between polymorphism and T2D with the OR = 3.28 (95% CI = 1.45-7.44, p = 0.003). This finding is in accord with that of Barra *et al.*[34], who also showed that the recessive model fitted the effect of rs7903146 (C/T) polymorphism of the TCF7L2 gene on the risk of T2D in the Brazilian population. Based on the findings, T allele and TT genotype are associated with the risk of diabetes mellitus in the Azerbaijan population. The number of TT genotypes in the case group was more than twice higher than that in the control group. This could be due to the small sample size and further studies with larger sample numbers will be required to confirm current data. Moreover, lifestyle, diet, smoking and other environmental factors could also explain the risk differences.

CONCLUSION

Current findings indicate that individuals who carry the TT genotype of rs7903146 polymorphism of TCF7L2 gene have a higher risk of T2D developing. Thus, rs7903146 (C/T) could be used for therapeutic purposes as a marker to predict the occurrence of T2D in the Azerbaijan population. Obtained data could help to estimate the probability of developing a T2D in the Azerbaijan population by designing the risk prediction models (diagnostic and prognostic models).

SIGNIFICANCE STATEMENT

This study discovered the strong association of the TCF7L2 gene polymorphism of rs7903146 (C/T) and T2D diabetes among the Azerbaijan population. The results of the study can be beneficial for the improvement of the health policy of the country as well as the creation of risk prediction models based on the genetic determinants of diabetes mellitus. Moreover, these results could be used in the meta-analysis studies and help the researchers to understand the distribution of this polymorphism among Caucasian and Asian countries.

ACKNOWLEDGMENT

This study was funded by the Institute of Molecular Biology and Biotechnologies, Azerbaijan National Academy of Sciences.

REFERENCES

1. Galicia-Garcia, U., A. Benito-Vicente, S. Jebari, A. Larrea-Sebal and H. Siddiqi *et al.*, 2020. Pathophysiology of type 2 diabetes mellitus. Int. J. Mol. Sci., Vol. 21. 10.3390/ijms21176275.
2. Grant, S.F., G. Thorleifsson, I. Reynisdottir, R. Benediktsson and A. Manolescu *et al.*, 2006. Variant of transcription factor 7-like 2 (*TCF7L2*) gene confers risk of type 2 diabetes. Nat. Genet., 38: 320-323.
3. Florez, J.C., 2007. The new type 2 diabetes gene TCF7L2. Curr. Opin. Clin. Nutr. Metab. Care, 10: 391-396.
4. da Silva Xavier, G., M.K. Loder, A. McDonald, A.I. Tarasov and R. Carzaniga *et al.*, 2009. TCF7L2 regulates late events in insulin secretion from pancreatic islet β-cells. Diabetes, 58: 894-905.
5. Müller, T.D., B. Finan, S.R. Bloom, D. D'Alessio and D.J. Drucker *et al.*, 2019. Glucagon-like peptide 1 (GLP-1). Mol. Metab., 30: 72-130.
6. Yi, F., P.L. Brubaker and T. Jin, 2005. TCF-4 mediates cell type-specific regulation of proglucagon gene expression by β-catenin and glycogen synthase kinase-3β. J. Biol. Chem., 280: 1457-1464.
7. Lyssenko, V., R. Lupi, P. Marchetti, S. Del Guerra and M. Orho-Melander *et al.*, 2007. Mechanisms by which common variants in the TCF7L2 gene increase risk of type 2 diabetes. J. Clin. Invest., 117: 2155-2163.

8. Holst, J.J., T. Vilsbøll and C.F. Deacon, 2009. The incretin system and its role in type 2 diabetes mellitus. Mol. Cell. Endocrinol., 297: 127-136.
9. Folsom, A.R., J.S. Pankow, J.M. Peacock, S.J. Bielinski, G. Heiss and E. Boerwinkle, 2008. Variation in TCF7L2 and increased risk of colon cancer: The atherosclerosis risk in communities (ARIC) study. Diabetes Care, 31: 905-909.
10. Prunier, C., B.A. Hocevar and P.H. Howe, 2004. Wnt signaling: Physiology and pathology. Growth Factors, 22: 141-150.
11. Giovannucci, E., 2001. Insulin, insulin-like growth factors and colon cancer: A review of the evidence. J. Nutr., 131: 3109S-3120S.
12. Kretzschmar, K. and H. Clevers, 2017. Wnt/β-catenin signaling in adult mammalian epithelial stem cells. Dev. Biol., 428: 273-282.
13. Shu, L., A.V. Matveyenko, J. Kerr-Conte, J.H. Cho, C.H.S. McIntosh and K. Maedler, 2009. Decreased TCF7L2 protein levels in type 2 diabetes mellitus correlate with downregulation of GIP- and GLP-1 receptors and impaired beta-cell function. Hum. Mol. Genet., 18: 2388-2399.
14. Shah, M., R.T. Varghese, J.M. Miles, F. Piccinini and C.D. Man *et al*., 2016. TCF7L2 genotype and α-cell function in humans without diabetes. Diabetes, 65: 371-380.
15. Slattery, M.L., A.R. Folsom, R. Wolff, J. Herrick, B.J. Caan and J.D. Potter, 2008. Transcription factor 7-like 2 polymorphism and colon cancer. Cancer Epidemiol. Biomarkers Prev., 17: 978-982.
16. Yazdi, K.V., S.M. Kalantar, M. Houshmand, M. Rahmanian and M.R. Manaviat *et al*., 2020. SLC30A8, CDKAL1, TCF7L2, KCNQ1 and IGF2BP2 are Associated with Type 2 Diabetes Mellitus in Iranian Patients. Diabetes, Metab. Syndrome Obesity: Targets Ther., 13: 897-906.
17. Mustafa, S. and D. Younus, 2021. Association of TCF7L2 RS7903146 polymorphism with the risk of type 2 diabetes mellitus (T2DM) among kurdish population in Erbil province, Iraq. Indian J. Clin. Biochem., 36: 312-318.
18. Dalhat, M., H. Bello, B. Ibrahim and A. Labbo, 2017. Association of rs7903146 TCF7L2 (C/T) gene polymorphism and type 2 diabetes mellitus in Pakistani population. J. Appl. Life Sci. Int., Vol. 14. 10.9734/jalsi/2017/37411.
19. Benberin, V., T. Voshchenkova, G. Abildinova, A. Borovikova and A. Nagimtayeva, 2020. Analysis of polymorphisms associated with type 2 diabetes in the Kazakh population. J. Mol. Genet. Med., Vol. 14. 10.37421/jmgm.2020.14.464.
20. Yuzbasiogullari, A.B., 2020. Sex-specific associations of TCF7L2 variants with fasting glucose, type 2 diabetes and coronary heart disease among Turkish adults. Anatolian J. Cardiol., 24: 326-333.
21. Dutra, L.A.S., P.G.G. Costa, L.F.R. Velasco, A.A. Amato and G.B. Barra, 2008. Allele-specific PCR assay to genotype SNP rs7903146 in TCF7L2 gene for rapid screening of diabetes susceptibility. Arquivos Brasileiros Endocrinologia Metabologia, 52: 1362-1366.
22. van Delden, J.J.M. and R. van der Graaf, 2016. International Ethical Guidelines for Health-related Research Involving Humans. 4th Edn., CIOMS, Geneva, Switzerland, 122.
23. Alsmadi, O., K. Al-Rubeaan, G. Mohamed, F. Alkayal and H. Al-Saud *et al*., 2008. Weak or no association of TCF7L2 variants with type 2 diabetes risk in an Arab population. BMC Med. Genet., Vol. 9. 10.1186/1471-2350-9-72.
24. Saadi, H., N. Nagelkerke, S.G. Carruthers, S. Benedict and S. Abdulkhalek *et al*., 2008. 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 Res. Clin. Pract., 80: 392-398.
25. Najwa, A.Sh., H.Y. Ali and D.I. Hanash, 2019. Polymorphism study of TCF7L2 gene and related to some biochemical parameters in DM2 females Iraqi patients. Res. J. Sci. Technol., 11: 1-8.
26. Ding, W., L. Xu, L. Zhang, Z. Han, Q. Jiang, Z. Wang and S. Jin, 2018. Meta-analysis of association between TCF7L2 polymorphism rs7903146 and type 2 diabetes Mellitus. BMC Med. Genet., Vol. 19. 10.1186/s12881-018-0553-5.
27. Bonora, E., F. Calcaterra, S. Lombardi, N. Bonfante, G. Formentini, R.C. Bonadonna and M. Muggeo, 2001. Plasma glucose levels throughout the day and HbA_{1c} interrelationships in type 2 diabetes: Implications for treatment and monitoring of metabolic control. Diabetes Care, 24: 2023-2029.
28. Yan, Y., K.E. North, G. Heiss, R. Klein and C.J. Girman *et al*., 2010. Transcription factor 7-like 2 (TCF7L2) polymorphism and context-specific risk of impaired fasting glucose in African American and caucasian adults: The atherosclerosis risk in communities (ARIC) study. Diabetes Metab. Res. Rev., 26: 371-377.
29. Avzaletdinova, D.S., L.F. Sharipova, O.V. Kochetova, T.V. Morugova, V.V. Erdman, R.S. Somova and O.E. Mustafina, 2016. The association of TCF7L2 rs7903146 polymorphism with type 2 diabetes mellitus among tatars of bashkortostan. Diabetes mellitus, 19: 119-124.
30. Hameed, T., Z. Khan, M. Imran, S. Ali, A.A. Albegali, M.I. Ullah and H. Ejaz, 2021. Associations of transcription factor 7-like 2 (TCF7L2) gene polymorphism in patients of type 2 diabetes mellitus from Khyber Pakhtunkhwa population of Pakistan. Afr. Health Sci., 21: 15-22.

31. Amoli, M.M., P. Amiri, J. Tavakkoly-Bazzaz, E. Charmchi and J. Hafeziyeh *et al.*, 2010. Replication of TCF7L2 rs7903146 association with type 2 diabetes in an Iranian population. Genet. Mol. Biol., 33: 449-451.
32. Barros, C.M.A.R., A.P. Araújo-Neto, T.R. Lopes, M.A.L. Barros and F.J.N. Motta *et al.*, 2014. 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: 7889-7898.
33. Tong, Y., Y. Lin, Y. Zhang, J. Yang, Y. Zhang, H. Liu and B. Zhang, 2009. 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., 10.1186/1471-2350-10-15.
34. Barra, G.B., L.A.S. Dutra, S.C. Watanabe, P.G.G. Costa, P.S.M. da Cruz, M.F. Azevedo and A.A. Amato, 2012. Association of the rs7903146 single nucleotide polymorphism at the transcription factor 7-like 2 (TCF7L2) locus with type 2 diabetes in Brazilian subjects. Arquivos Brasileiros Endocrinologia Metabologia, 56: 479-484.