Genomic study of TCF7L2 gene mutation on insulin secretion For Type 2 DM Patients – Critical review

Abstract:

Background: Diabetes type 2 is the most common metabolic disorder worldwide. Beta cell dysfunction reduces insulin secretion and increases the glucose level in the blood and insulin resistance that raises the glucose production in the liver and decreases the glucose uptake to muscle, liver, and adipose tissue causing hyperglycemia (T2DM). TCF7L2 (transcription factor 7–like 2) works as a nuclear Receptor for CTNNB1(B catenin) that mediated the WNT signaling pathway (a group of signal transduction pathways made of proteins that pass signals from outside a cell through cell surface receptors to the inside of the cell) and any variation will cause the development of T2DM.

Method: GenBank in NCBI database was used to extract the DNA sequence and mRNA sequence of the TCF7L2 gene (an accession number of the gene, number of amino acids, exons, and length of nucleotides). FASTA format was also useful to retrieve the nucleotide sequence and get the function of the protein. BLAST was used to compare the protein product of the TCF7L2gene between humans and gorillas**,** and pygmy chimpanzees **(**Pan paniscus).

Results: The accession number is NC_000010.11, the number of amino acids in the protein product is 602, the number of exons found is 20 and the gene is in chromosome 10. Finally, many organisms have the same gene as dogs, cows, mice, rats, zebrafish, and frogs.

Conclusion: There is a strong association between TCF7L2 (transcription factor 7–like 2) alleles (rs7903146) T alleles and T2DM**.** It was found that there is a high frequency of diabetic type two patients having TCF7L2 (transcription factor 7–like 2) alleles (rs7903146) with a high frequency of the T allele.

Keywords: TCF7L2 (transcription factor 7–like 2), T2DM (Type 2 Diabetes Mellitus).

Introduction:

Type 2 diabetes considers for more than 90% of patients with diabetes. The uncontrolled type 2 diabetes can cause serious problems including heart disease and stroke.

According to a study done by Khan et al in 2019, 462 million individuals are suffering from type 2 diabetes, corresponding to 6.28% of the total population in the world. In 2017, more than one million deaths, and it is ranked as the ninth cause of mortality. (Khan et al., 2019). Diabetes type 2 is the most common metabolic disorder worldwide. Beta cell dysfunction causes a low level of insulin secretion and increases the glucose level in the blood. Insulin resistance has a role in glucose production in the liver and reduces the glucose uptake to muscle, liver, and adipose tissue. Also, if both present beta cell dysfunction and insulin resistance, it will lead to hyperglycemia that developed to type 2 diabetes. (Galicia-Garcia et al., 2020).

The risk factor associated with type 2 diabetes Mellitus includes low physical activity, smoking, alcoholic intake, and obesity (Kolb & Martin, 2017). Moreover, the genetic component has affected the development of T2DM, as a study shows a higher rate of 96% in monozygotic compared to dizygotic twins. Also, first-degree relatives have a higher incident (40%) compared to the general population (6%). Additionally, some susciptlity loci have related to T2DM. for example, KCNJ11 (potassium inwardly-rectifying channel, subfamily J, member 11), TCF7L2 (transcription factor 7–like 2, IRS1 (insulin receptor substrate 1), MTNR1B (melatonin-receptor gene), PPARG2 (peroxisome proliferator activated receptor gamma 2), IGF2BP2 (insulin-like growth factor two binding protein 2), CDKN2A (cyclin-dependent kinase inhibitor 2A), HHEX (haematopoietically expressed homeobox) and FTO (fat mass and obesity-associated) gene. (Wu et al., 2014).

TCF7L2 (transcription factor 7–like 2) works as a nuclear Receptor for CTNNB1(B catenin) that mediated the WNT signaling pathway. This protein plays a role in many disorders including (Latent autoimmune diabetes in adults (LADA), Gestational diabetes mellitus (GDM), obesity, Small Bowel Syndrome, Crohn's Disease (CD), Diabetic Nephropathy (DN), gastric cancer, prostatic cancer, breast cancer, Clear-cell renal cell carcinoma (CCRCC), Schizophrenia, Cystic Fibrosis, cardiovascular disease, endocrine disorders and Polycystic ovarian syndrome (PCOS). (Bosque-Plata et al., 2021).

TCF7L2 had many variants associated with T2DM most commonly rs7903146 and rs12255372 (del Bosque-Plata et al., 2021). In this study, we aimed to find out the relation between TCF7L2 (rs7903146) variant and its relation to type 2 diabetes mellitus.

Method:

For better understanding TCF7L2 gene, we used computational techniques programs to analyze the gene. NCBI database was used to retrieve genomic information. we found that the accession number of genes is NC_000010.11, the number of amino acids is 602 the number of exons is 20, the nucleotide length is 217,431 nt and nucleotides location (112950247..11316767).

To find out the nucleotide sequence of mRNA in TCF7L2 gene, we used FASTA format, the most basic format for reporting a sequence and is accepted by almost all sequence analysis program.

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>NM_001146274.2 Homo sapiens transcription factor 7 like 2 (TCF7L2),
transcript variant 1, mRNA
GTCAATAATCTCCGCTCCCAGACTACTCCGTTCCTCCGGATTTCGATCCCCCTTTTTCTATCTGTCAATC
AGCGCCGCCTTTGAACTGAAAAGCTCTCAGTCTAACTTCAACTCACTCAAATCCGAGCGGCACGAGCACC
TCCTGTATCTTCGGCTTCCCCCCCCCTTTGCTCTTTATATCTGACTTCTTGTTGTTGTTGGTGTTTTTTT
TTTTTTTACCCCCCTTTTTTATTTATTATTTTTTTGCACATTGATCGGATCCTTGGGAACGAGAGAAAAA
AGAAACCCAAACTCACGCGTGCAGAAGATCTCCCCCCCCCTTCCCCTCCCCTCCTCCCTCTTTTCCCCTCC
CCAGGAGAAAAAGACCCCCAAGCAGAAAAAAGTTCACCTTGGACTCGTCTTTTTCTTGCAATATTTTTTG
GGGGGGCAAAACTTTTTGGGGGTGATTTTTTTTGGCTTTTCTTCCTCCTTCATTTTTCTTCCAAAATTGC
TGCTGGTGGGTGAAAAAAAAATGCCGCAGCTGAACGGCGGTGGAGGGGATGACCTAGGCGCCAACGACGA
ACTGATTTCCTTCAAAGACGAGGGCGAACAGGAGGAGAAGAGCTCCGAAAACTCCTCGGCAGAGAGGGAT
TTAGCTGATGTCAAATCGTCTCTAGTCAATGAATCAGAAACGAATCAAAACAGCTCCTCCGATTCCGAGG
CGGAAAGACGGCCTCCGCCTCGCTCCGAAAGTTTCCGAGACAAATCCCGGGAAAGTTTGGAAGAAGCGGC
CAAGAGGCAAGATGGAGGGCTCTTTAAGGGGCCACCGTATCCCGGCTACCCCTTCATCATGATCCCCGAC
CTGACGAGCCCCTACCTCCCCAACGGATCGCTCTCGCCCACCGCCCGAACCCTCCATTTTCAGTCCGGCA
GCACACATTACTCTGCGTACAAAACGATTGAACACCAGATTGCAGTTCAGTATCTCCAGATGAAATGGCC
ACTGCTTGATGTCCAGGCAGGGAGCCTCCAGAGTAGACAAGCCCTCAAGGATGCCCGGTCCCCATCACCG
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The nucleotide sequence in FASTA format for the TCF7L2 gene is

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>NC_000010.11:112950247-113167678 Homo sapiens chromosome 10, GRCh38.p14
Primary Assembly
GTCAATAATCTCCGCTCCCAGACTACTCCGTTCCTCCGGATTTCGATCCCCCTTTTTCTATCTGTCAATC
AGCGCCGCCTTTGAACTGAAAAGCTCTCAGTCTAACTTCAACTCACTCAAATCCGAGCGGCACGAGCACC
TCCTGTATCTTCGGCTTCCCCCCCCCTTTGCTCTTTATATCTGACTTCTTGTTGTTGTTGGTGTTTTTTT
TTTTTTTACCCCCCTTTTTTATTTATTATTTTTTTGCACATTGATCGGATCCTTGGGAACGAGAGAAAAA
AGAAACCCAAACTCACGCGTGCAGAAGATCTCCCCCCCCCTTCCCCTCCCCTCCTCCCTCTTTTCCCCTCC
CCAGGAGAAAAAGACCCCCAAGCAGAAAAAAGTTCACCTTGGACTCGTCTTTTTCTTGCAATATTTTTTG
GGGGGGCAAAACTTTTTGGGGGTGATTTTTTTTGGCTTTTCTTCCTCCTTCATTTTTCTTCCAAAATTGC
TGCTGGTGGGTGAAAAAAAAATGCCGCAGCTGAACGGCGGTGGAGGGGATGACCTAGGCGCCAACGACGA
ACTGATTTCCTTCAAAGACGAGGGCGAACAGGAGGAGAAGAGCTCCGAAAACTCCTCGGCAGAGAGGGAT
TTAGCTGATGTCAAATCGTCTCTAGTCAATGAATCAGAAACGAATCAAAACAGCTCCTCCGATTCCGAGG
TAGGAAAAGCCCCTCGGGCTGGTGGGGTTTTTTATCTGTTTCCTGGGCTTGGCAAATGTTGCTGAAAGGG
GAGAAATCGGGGCTGGGGGCGGCGGCGGGGCCCGGCGGGCGGCGTGTGCGTACGGTGCCACCATTGCAAA
AACTTGTAACCCTGTTTTTTTCTACCCCCCCCTCGACCTCGCCGATTCTTTTTCTCCCCCTTCTCCCCCT
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The TCF7L2 gene is in chromosome 10**.** it is also known as TCF4 or TCF-4. This gene encodes a high mobility group (HMG) box-containing transcription factor which plays important role in the Wnt signaling pathway. This protein is involved in blood glucose homeostasis. Genetic variants of this gene are associated with an increased risk of type 2 diabetes. Several transcript variants encoding multiple different isoforms have been found for this gene. The SLC23A2 gene is conserved in chimpanzees, Rhesus monkeys, dogs, cows, mice, rats, chickens, zebrafish, thaliana, rice, and frogs.

There is homology between the TCF7L2 gene and western lowland gorilla**,** and pygmy chimpanzee **(**Pan paniscus) with 100% identity and 0% gaps.

Results and Discussion:

Article No.	Article Title	Author/Year	No.T2DM	Control No.	Genotype distribution			Allelic distribution	
1	Transcription factor 7-like-2 (*tcf7l2*) RS7903146 (C/T) polymorphism in patients with type 2 diabetes	Bahaaeldin, Seif, Hamed, & Kabiel, 2020	70	30	CC P: 39% C: 46.7%	CT P: 57.1% C: 53.3%	TT P: 4.3% C: 0 %	C P: 67.1% C: 73.3%	T P: 32.9% C: 26.7%
2	Association of TCF7L2 RS7903146 polymorphism with the risk of type 2 diabetes mellitus (T2DM) among Kurdish population in Erbil Province, Iraq	Mustafa & Younus, 2020	106	106	p: 24.5% C: 45.3%	P: 69.8% C: 50.9%	p: 5.7% C: 3.8%	p: 59.4% C: 70.8%	P: 40.6% C: 29.2%
3	Association of the RS7903146 single nucleotide polymorphism at the transcription factor 7-like 2 (TCF7L2) locus with type 2 diabetes in Brazilian subjects	Barra et al., 2012	113	139	P: 43.4% C: 50.4%	P: 41.6% c: 45.6%	P: 15% C: 4.3%	P: 64.2% C: 73%	P: 35.8% C: 4.3%
4	Significant	Elhourch et	150	100	P:	P:	P:	P:	P:

	association of polymorphisms in the TCF7L2 gene with a higher risk of type 2 diabetes in a Moroccan population	al., 2021			33.3% C: 36%	42.7% C: 59%	24% C: 5%	54.8% C: 65.5%	45.2% C: 34.5%
5	Associations of transcription factor 7-like 2 (TCF7L2) gene polymorphism in patients of type 2 diabetes mellitus from Khyber Pakhtunkhwa population of Pakistan		118	58	P: 15.2% C: 41.3%	P: 75.4% C: 46.5%	P: 9.3% C: 12%	p: 52% C: 64%	P: 47% C: 35%

Table 1: Results of review research.

In this study, we are investigating the relatively frequently studied association between TCF7L2 (transcription factor 7–like 2) alleles (rs7903146) and T2DM.

Pathogenicity of TCF7L2 (transcription factor 7–like 2) alleles (rs7903146):

TCF7L2 (transcription factor 7–like 2) works as a nuclear Receptor for CTNNB1(B catenin) that mediated the WNT signaling pathway and play important role in the secretion of Glucagon Like Peptide-1 (GLP-1) produced by intestinal endocrine L cell. So, any changes in the WNT pathway will lead to reducing GLP-1 secretion which will affect the insulin secretion and generation of B cells. Furthermore, TCF7L2 activates mRNA expression of glucagon and GLP-1 in the gut endocrine cell by stimulation of insulin secretion, inhibition of glucagon, and enhancement of peripheral insulin sensitivity and induction of repletion. Therefore, any alternation of TCF7L2 will cause T2DM. (Gupta et al., 2008). These had been confirmed by many clinical trials.

One of them studied the association between the TCF7L2 variant (rs7903146) and the mechanism by which this gene affects type 2 diabetes. The research trial used the Incretin effect in the TCF7L2 variant. The incretin effect compares the insulin secretion when glucose is taken orally or intravenously. The study shows that the incretin effect reduced by 30% in response to

the overall B-cell responsivity tends to lower in risk group TCF7L2 (rs7903146) genotypes CC and TC by 50 during OGTT.

Also, it shows normal GIP-1 AND GIP in Both group at-risk and Non-at risk TCF7L2 genotypes. So, this concludes the inability of pancreatic B-cells in the TCF7L2 genotype to respond to the normal concentration of GIP-1 and GIP. In contrast, the TCF7L2 genotype does not significantly affect insulin response to intravenous glucose because it shows normal b-cell dose response. (Villareal et al., 2009)

Another trial investigated the Postprandial glucose metabolism of T2DM patients carrying TCF7L2 alleles (rs7903146). At the beginning of the study, all members have similar Impaired Fasting glucose and Impaired glucose tolerance. All members have similar Fasting Plasma glucose, Insulin C peptide, and also Fasting Endogenous glucose production. The only difference is Plasma Triglycerides in T alleles are lower compared to CC individuals. After MMT, the plasma glucose level and insulin secretion in the TT subject were reduced compared to the CC group.

The peak of insulin Secretion rate was higher in the CC group and it is not connected to any changes in B cell sensitivity. Additionally, reduced level of Ra ex (rate of entry of meal-derived exogenous glucose into the Systemic circulation) in The carrier of risk T allele because of decreased intestinal glucose absorption and increase glucose reservation in the spleen.

Moreover, the level of GLP-1 and GIP level were similar in all three groups. (Daniele et al., 2015).

TCF7L2 (transcription factor 7–like 2) alleles(rs7903146) (C/T) and T2DM:

Many trials confirmed that TCF7L2 (transcription factor 7–like 2) alleles (rs7903146) (C/T) increase the risk of T2DM. See table 1.

In the first article, a case-control study was conducted in Egypt to investigate the association between TF7L2 rs7903146 (C/T) gene polymorphism in patients with T2DM. Biochemical

analysis of the patient group shows a significant increase in HBA1C, serum triglycerides, LDL-C, and TC. In contrast, HDL-C was higher in the control group than in patients.

As the results of qRT-PCR of the test, the group shows that wild-type genotype CC(39.6%), the heterozygous CT genotype(57.1%), and the mutant TT genotype (4.3%). In the controls, 46.7% were CC genotype, CT genotype (53.3%), and no TT genotype. This concludes that the minor T allele of the rs7903146(C/T) SNP was associated with a T2DM. See table1 (Bahaaeldin, Seif, Hamed, & Kabiel, 2020).

A further clinical trial was done among Kurdish ethnicity in Iraq using Tetra-primer ARMS-PCR assay. It aimed to study the relationship between the TCF7L2 rs7903146 (C /T) variant and T2DM. The patients had elevated BMI(P value = 0.02), HbA1c(P value=0.008), TC(P value=0.006), TG (P value=0.009and LDL cholesterol (P value=0.007) compared to the control groups. Furthermore, serum insulin and Fasting blood sugar indicated higher results in participants compared to the control group (P value = 0.01). Whereas HDL cholesterol was significantly higher in the controls group compared to the patients (P value = 0.02), the genotypic and allelic frequencies of rs7903146(CC, CT, and TT) in the Kurdish population shows that CC (Test= 24.5%, control= 45.3%), CT (Test= 69.8%, control= 50.9%) and TT (Test= 5.7%, control= 3.8%). The frequency of C alleles is higher in the control group compared to the diabetic; 70.8%, and 59.4% respectively, while the T alleles are more frequent in the diabetic group compared to control subjects; 40.6% and 29.2% respectively. The T allele could be a risk factor for increasing the risk of T2DM rate in the Iraqi Kurdish population.see table 1 (Mustafa & Younus, 2020).

Case-control trial was performed among Brazilian subjects to investigate the relationship between the T allele of the single nucleotide polymorphism (SNP) rs7903146 of TCF7L2 with the occurrence of T2D. using alleles-specific PCR. The results shows that genotyping frequency that CC (Test= 43.4% , control =50.4%), CT (Test= 41.6%, control=45.6%) and TT (Test=15%, control=4.3%). The frequency of C alleles in control subjects was 73% which is higher compared to the patients' group 64.2%. On the other hand, T2DM patients have a slightly higher frequency of T alleles about 35.8% compared to control subjects 27%. Therefore, the T alleles of (SNP)

rs7903146 of TCF7L2 is significantly associated with type 2 diabetes mellitus. See table 1 (Barra et al., 2012)

According to research was done by Elhourch et al., the Moroccan population shows that the frequency of rs7903146 genotypes in diabetes subjects (CC: 33.3%, CT: 42.7%, TT: 24.0%), whereas in the control group was (CC:36%, CT:59%, TT:5%). So, the TT genotype is associated with an increased risk of T2DM. Additionally, The frequency of the C allele in the control group is 65.5% while in diabetic patients is 54.8% which is not significantly different in both groups. Besides, the T allele was more frequent in diabetic subjects compared to the control group (45.2%, 34.5% respectively). (Elhourch et al., 2021). See table 1

A study done in Khyber Pakhtunkhwa of Pakistan indicates that SNPs of the TCF7L2 rs7903146 gene are significantly associated with T2DM disease. The research shows that SNPs rs7903146 heterozygous CT is higher in diabetic participants compared to control ($p < 0.0001$). Also, T alleles are more in cases than in control subjects ($p<0.000$). (Hameed et al., 2021) see table 1

Factors affect the TCF7L2 (transcription factor 7–like 2):

Many factors affect the frequency of genetic variation of TCF7L2 including lifestyle. In a systematic review, the study includes thirty-eight study mention that high dietary intake with high levels of carbohydrates and fat can increase glucose concentration among TT alleles carriers. also, BMI can reduce the high glucose level in the blood but there was multiple conflicting research concerning physical activity and smoking. (Hosseinpour-Niazi et al., 2022).

The genetic frequency can also be different between different ethnic groups that have been mentioned in many types of research one of them in southwestern Iran includes 150 T2DM patients and 150 healthy individuals. The case-control indicates no association between T2 DM and the rs7903146 variant. (Foroughmand et al., 2019). On contrary, another study in Iran shows a strong association between T2DM and rs7903146 and it proves that could be affected by different ethnic groups and different lifestyles. (Amoli et al., 2010).

Conclusion:

The results of our critical review are based on African, Asian, and Brazilian subjects. It is composed of 557 diabetic patients and 433 control subjects. All five articles confirm the strong association between TCF7L2 (transcription factor 7–like 2) alleles(rs7903146) T alleles and T2DM. Further research is required with a large-scale population of a different ethnic group to confirm this association.

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