Original Research

Evaluation of rs7903146 Polymorphism in TCF7L2 Gene among Type 2 Diabetes Patients

Salar Momen Maragheh[1], Alireza Aslani Zakariya[2], Elham Ghaedi[3], Fahimeh Saeifar[4]
Mahdi Navid Talemi[5], Mehran Rezanezhad Varjovi[6], Mahdi Bagheri[7*]

1. Biotechnology Research Center (BRC), Pasteur Institute of Iran, Tehran, Iran, Department of Biotechnology, Islamic Azad University, Central Tehran Branch, Tehran, Iran
2. Department of Chemistry, Faculty of Science, Ataturk University, Erzurum, 25240, Turkey
3. Department of Medicine, Pirogov Russian National Research Medical University, Moscow, Russia
4. Department of Midwifery, Health Center of Maragheh, Maragheh, East Azerbaijan, Iran, Department of Genetics, Islamic Azad University of Bonab, Bonab, East Azerbaijan, Iran
5. Department of Pharmacology, Guilan University of Medical Sciences, Rasht, Guilan, Iran
6. Department of Microbiology, Islamic Azad University of Maragheh, Maragheh, East Azerbaijan, Iran
7. Department of Medicine, Medical University of Ilam, Ilam, Iran

***Corresponding Author: Mahdi Bagheri**, Department of Medicine, Medical University of Ilam, Ilam, Iran. Email:mahdibagheri121995@gmail.com. Orcid: 0000-0001-9707-5719.

Abstract:

Background:

Diabetes is characterized by decreased insulin sensitivity and is a growing health threat worldwide. Therefore, this study aimed to evaluate the relationship between rs7903146 polymorphism in the TCF7L2 gene with type 2 diabetes in East Azerbaijan province, Iran.

Materials and method:

A total of 100 blood samples were collected from diabetic and healthy people. The samples were subjected to specific primers, PCR, and electrophoresis after DNA extraction from all samples and quality control. Finally, the PCR products were treated with RSal restriction enzyme and electrophoresed again, and the target polymorphism was checked.

Results:

The frequency of genotypes were TT=33.7%, CC=16.8% and CT=49.5% for healthy people and TT=43%, CC=14% and TC=43% in diabetics. The percentage of the T allele in healthy and diabetic people was as much as 42.1% and 64.5%, respectively.

Conclusion:

According to the results, there was probably a relationship between rs7903146 polymorphism in the TCF7L2 gene and diabetes.

Keywords: Polymorphism, rs7903146, TCF7L2, Type 2 diabetes

Submitted: 2 November 2022, Revised: 13 November 2022 , Accepted: 25 November 2022

Introduction

The metabolic disease of diabetes is characterized by chronic blood sugar increases and disturbances in fat and protein metabolism caused by defects in insulin secretion, insulin action, or both (1). Generally, diabetes is divided into four groups, type 1 diabetes, type 2 diabetes, gestational diabetes, and diabetes of various causes, among which type 2 diabetes is the most common type of diabetes, including (2).

In type 2 diabetes, glucose cannot be used or stored by the body, so instead of being converted into energy, it returns to the bloodstream and causes symptoms. The cause of type 2 diabetes is a combination of lifestyle and genetic factors. While a person can control some of these issues such as diet and obesity, other issues such as aging, being female, and genetics, cannot be controlled. However, the exact cause of this disease has not yet been determined, and its prevalence rate is also increasing. In the last decade, the prevalence of this disease has increased alarmingly in Iran (3,4).

Evidence shows that common and rare genetic polymorphisms can affect the risk of diabetic complications (5). Molecular genetic factors associated with diabetes are difficult to recognize due to their extent, complexity, and multifactorial nature. Most people with diabetes have many genes, each of which can play a small role in increasing the risk of developing type 2 diabetes. The number of genes contributing to type 2 diabetes has increased to more than 36 since 2011 (6). All these genes together constitute only 10% of the total hereditary component of this disease. For example, the TCF7L2 gene sequence increases the risk of developing diabetes up to 1.5 times as the most extensive common genetic risk. Single nucleotide polymorphism 7903146rs is located in intron number 3 of the TCF7L2 gene, significantly affecting type 2 diabetes (7). The expression of TCF7L2 in pancreatic cells of type 2 diabetic patients compared to healthy individuals was reported to be accompanied by a decrease in insulin secretion. Several studies have also reported a decrease in insulin secretion in response to increased expression of TCF7L2 in the pancreas, apart from the effect of the TCF7L2 variant on cell structure and insulin release from pancreatic beta (8). Previous studies have indicated that some single nucleotide polymorphisms (SNP) in the TCF7L2 gene were strongly related to the risk of T2DM in an Icelandic case-control sample. This study aimed to evaluate the relationship between rs7903146 polymorphism in the TCF7L2 gene in patients with type 2 diabetes in East Azerbaijan province, Iran.

Materials and method

This study was conducted on 101 patients whose information was recorded in a questionnaire. In addition, 101 healthy people from the general population were selected as the control group. The inclusion criteria were no family history of type 2 diabetes, at least in the previous two generations, and a negative test for glucose tolerance test. The control group was age-matched with the patients. In this study, about 2cc of blood samples were taken from each person and collected in tubes containing EDTA. The collected samples were transferred to the laboratory with a flask dry ice and kept at -20°C until extraction. Tubes containing blood samples of patients and controls were numbered separately, and their gender was also mentioned on the tubes. The extraction kit of AMINSAN company was used to extract genomic DNA, and the extraction steps were carried out based on the instructions of the relevant kit. PCR process of blood samples was performed using recommended methods. DNA fragments were observed using the electrophoresis of PCR products. The PCR products were treated with the restriction enzyme Rsal, the gel was transferred to the transilluminator, and the DNA fragment bands

were cut, observed, and photographed. The sequence of the primers used is presented in Table 1.

Data analysis

The data were analyzed using SPSS version 22 software after completing the experiments. Genotype distribution was calculated for the studied SNPs, and Hardy-Weinberg equilibrium and chi-square test with one degree of freedom (df) were used to determine the deviation.

Results

The average age of the participants in this study (39.3% male and 60.7% female) was 52 years. There were 37% healthy male samples and 63% diabetic male samples, while there were 18% healthy female samples and 82% of diabetic female samples. Electrophoresis gel was placed under the influence of electric current from the negative to positive pole to determine DNA fragments. PCR was used to amplify single or small copies of a DNA fragment with a specific sequence. The applied ladder was 50 base pairs. The desired fragment length was 188 base pairs, formed between the bands of 150 and 200 base pairs of the ladder. The steps of PCR product in the samples of people with diabetes were similar to those of healthy people. The used ladder is 50 base pairs. The desired fragment length was 188 base pairs, formed between the bands of 150 and 200 base pairs of the ladder. The electrophoresis results of PCR products of patient samples showed that the length of the desired fragment was 188 base pairs, formed between the bands of 150 and 200 base pairs of the leader and used 50 base pairs of the leader. The electrophoresis results of the PCR product treated with Rsal enzyme in healthy individuals revealed that the TT polymorphism showed a band in 188 bp, while the TC polymorphism had a band in 159-188-29. Cutting CC gives band s29 and 159, and 29 is rarely seen. PCR products obtained from DNA extracted from blood samples of people with diabetes were treated with the restriction enzyme Rsal. The steps of the electrophoresis technique performed for healthy people were repeated for the patient samples. The three types of genotypes in this research included TT, CC, and TC, whose frequencies were investigated in the target population consisting 101 healthy and diabetic people. The results showed that the frequency of TT, CT, and CC genotypes in healthy people was 34, 17, and 50 cases, respectively, and the frequency of T, CT, and CC genotypes in diabetic people was 43, 43, and 14 cases, respectively (Figure 1).

According to data analysis in the healthy group, frequency of TT is 33.7% (34 samples), CT is 16.8% (17 samples), CC is 49.5% (50 samples), and the most frequent polymorphism in all samples is CC (50%). Separately, in the patient group, the frequency of TT is 43% (43 samples), CC 14% (14 samples), and CT 43% (43 samples), and the most frequent polymorphism in all samples is TT. Allele frequencies and percentages were calculated using genotype frequencies, as shown in Table 2.

The frequency of alleles in the target population showed that the T allele was the most frequent in diabetic people and showed a 22% increase compared to healthy people. The C allele was the most frequent in healthy people and showed a 22% decrease compared to diabetic people. These comparisons probably show a relationship between the increase of the T allele and diabetes (Figure 2).

Discussion

Given the role of genetic factors in developing type 2 diabetes, it seems necessary to investigate genes related to this disease.

The frequency of genotypes were TT=33.7%, CC=16.8% and CT=49.5% for healthy people and TT=43%, CC=14% and TC=43% in diabetics. Several studies reported the role of

TCF7L2 gene polymorphism (rs7903146) in a range of diseases consistent with the present study. In addition, the relationship between the TCF7L2 gene polymorphism (rs7903146) and the possibility of some diseases was also investigated. Abbas et al. (9) showed that TCF7L2 gene polymorphism (rs7903146) might play a role in the pathogenesis of diabetic nephropathy and co-morbidities (hypertension and dyslipidemia) in T2DM, which was in line with the results of the present study (9).

Other genes related to diabetes have also been investigated regarding type 2 diabetes, in addition to TCF7L2 gene polymorphism (rs7903146). Ranjbar et al. showed that the gene called CXCL5 (neutrophil-activating peptide from epithelial cells) is involved in the occurrence of cardiovascular diseases and some other diseases (10). This study is consistent with the results of the present study in terms of its effect on type 2 diabetes. However, Sari (11) indicated that GFPPT2 gene polymorphism is related to the risk of type 2 diabetes, and there is a significant difference in the frequency of TT genotype in patients with type 2 diabetes (11). The different results may be due to the difference in the examination of two different genes. The location of the GFPPT2 gene and its action mechanism differs from the TCF7L2 gene, but both are effective in diabetes. In another similar study, the results showed that several T alleles rs12255372, rs7903146, and rs290487 of TCF7L2 indicate the susceptibility to T2DM in the Kurdish population of Iran (12). Shokohi et al. investigated the relationship between rs7903146, rs12255372, and rs290487 polymorphisms in TCF7L2 gene with type 2 diabetes and concluded that T allele of rs7903146, rs12255372, and rs290487 polymorphisms of the TCF7L2 gene confers susceptibility to type 2 diabetes in Iranian Kurdish population (12). These results were consistent with those of this study.

Previous studies have revealed that TCF7L2 gene polymorphism (rs7903146) is related to type 2 diabetes, and this polymorphism is an important genetic risk factor for the development and progression of type 2 diabetes. Therefore, TCF7L2 polymorphism screening can lead to disease prognosis, disease progression prevention, and using treatment strategies to increase life expectancy and improve quality of life in patients with type 2 diabetes. This result is limited to the present study population, and more studies are needed on a larger number of people and different races to confirm the relationship between TCF7L2 polymorphism and type 2 diabetes.

Conclusion

According to the results, types of polymorphisms are a factor for genetic diversity and determining the phenotypic diversity between people, which may indicate a significant relationship in the susceptibility of people to diseases, progress of diseases, and genetic polymorphisms in the possibility of diabetes.

References

1. Rother, K. I. 2007. Diabetes Treatment--Bridging the Divide. The New England Journal of Medicine, 356, 1499-1501.
2. Pasquier, F. 2010. Diabetes and Cognitive Impairment: How to Evaluate the Cognitive Status? Diabetes & Metabolism, 36, S100-S105.
3. Lyssenko, V., Lupi, R., Marchetti, P., Del Guerra, S., Orho-Melander, M., Almgren, P., Sjögren, M., Ling, C., Eriksson, K.-F., Lethagen, Y.-L., Mancarella, R., Berglund, G., Tuomi, T., Nilsson, P., Del Prato, S. & Groop, L. 2007. Mechanisms By Which Common Variants In The Tcf7l2 Gene Increase Risk Of Type 2 Diabetes. The Journal of Clinical Investigation, 117, 2155-2163.

4. Bao, W., Hu, F. B., Rong, S., Rong, Y., Bowers, K., Schisterman, E. F., Liu, L. & Zhang, C. 2013a. Predicting Risk of Type 2 Diabetes Mellitus with Genetic Risk Models On The Basis Of Established Genome-Wide Association Markers: A Systematic Review. Am J Epidemiol, 178, 1197-207.
5. Fan, Z., Cai, Q., Chen, Y., Meng, X., Cao, F., Zheng, S. & Guo, J. 2016. Association Of The Transcription Factor 7 Like 2 (Tcf7l2) Polymorphism With Diabetic Nephropathy Risk: A Meta-Analysis. Medicine, 95, E3087-E3087.
6. Herder, C. & Roden, M. 2011. Genetics of Type 2 Diabetes: Pathophysiologic and Clinical Relevance. Eur J Clin Invest, 41, 679-92.
7. Mohammad, S., Abas Mohammad, P., Mehdi Abasi, R., Mehran Rajabi Sahneh, S. & Mostafa, D. 2019. Investigating the Distribution of Tcf7l2 (Rs7903146) Gene Polymorphism in Type 2 Diabetic Patients in West of Iran Mazandaran. Ilam University of Medical Science, 26, 156-163.
8. Elbein, S. C., Chu, W. S., Das, S. K., Yao-Borengasser, A., Hasstedt, S. J., Wang, H., Rasouli, N. & Kern, P. A. 2007b. Transcription Factor 7-Like 2 Polymorphisms and Type 2 Diabetes, Glucose Homeostasis Traits and Gene Expression in Us Participants of European and African Descent. Diabetologia, 50, 1621-30.
9. Abbas, S. A. N., Raza, S. T., Mir, S. S., Siddiqi, Z., Zaidi, A., Zaidi, Z. & Mahdi, F. 2019. Association of Variants Rs7903146 and Rs290487 of Tcf7l2 Gene with Diabetic Nephropathy and Co-Morbidities (Hypertension and Dyslipidemia) In Type 2 Diabetes Mellitus. Meta Gene, 20, 100561.
10. Ranjbar, H., Shirin, Amiri, Parvin, Namachian, Heshmat, Ramin, Sajjadi, Mirzaei and Rezazadeh. 2009. Investigating the relationship between Cxcl5 gene polymorphism and diabetes. Iranian Journal of Diabetes and Metabolism, 8, 247-252.
11. Sari, S. 2017a. Investigation of the Association between Gfpt2 Gene Polymorphism and Risk of Type 2 Diabetes. Qom Univ Med Sci J, 11, 64-69.
12. Shokouhi, S., Delpisheh, A., Haghani, K., Mahdizadeh, M. & Bakhtiyari, S. 2014. Association of Rs7903146, Rs12255372, and Rs290487 Polymorphisms in Tcf7l2 Gene with Type 2 Diabetes in An Iranian Kurdish Ethnic Group. Clin Lab, 60, 1269-76.

Tables

Table 1. Sequences of primers

Primer name	Primer sequence	Length (base pair)
Forward	5'-ACA ATT AGA GAG CTA AGC ACT TTT TAG GTA -3'	26
Reverse	5'-GTG AAG TGC CCA AGC TTC TC -3'	19

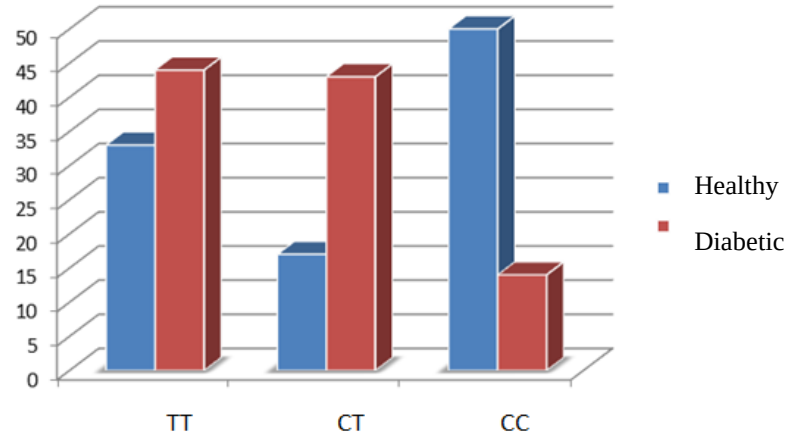

Figure 1. Frequency percentage of genotypes separately in healthy and diabetic samples

Table 2. The percentage of studied alleles and their percentage difference in healthy and diabetic people

Allele name	Allele percentage in diabetic people	Allele percentage in healthy people	Percentage difference between healthy and diabetic people	Chi-square	P
T	64.5 percent (129)	42.1 percent (85)	22%	20.293	0.000
C	35.5 percent (71)	57.9 percent (117)	22%		
Total	100%	100%			

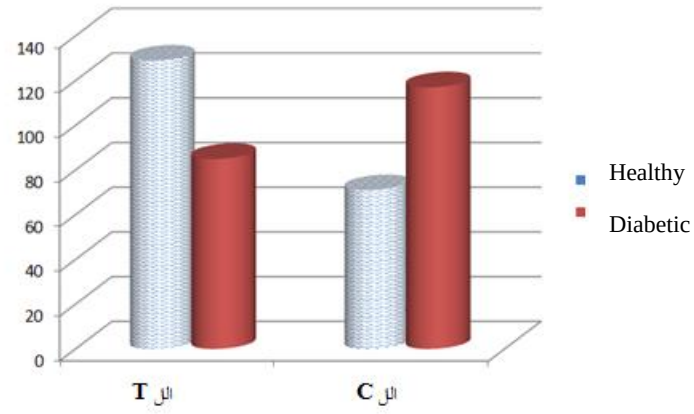

Figure 2. Frequency of alleles in the target population