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Relation of Rs12255372 (G/T) Polymorphisim in Transcription Factor 7 Like 2Gene with Betatrophin Level in Patients with Diabetes Mellitus Type 2

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Abstract

Transcription Factor7-Like2 also known as TCF7L2 or TCF4 is a protein acting as a transcription factor in human is encoded by the TCF7L2 gene. TCF7L2's role in glucose metabolism is expressed in many tissues such as gut, brain, liver and skeletal muscle. However,TCF7L2 does not directly regulate glucose metabolism in β-cell, but regulate glucose metabolism in pancreatic and liver.

This study aims to examine the role of rs12255372 (G/T) single nucleotide polymorphism of TCF7L2 gene polymorphism on the susceptibility of type 2 of Diabetes Mellitus (T2DM) patients, as well as to investigate the role of betatrophin in T2DM pathogenesis and to study the relation between rs12255372(G/T) polymorphism with betatrophin level in patients with T2DM and healthy control in Babylon Province.

The study was conducted from the period from November 2019 to July 2020. This case-control study include 90 subjects. 45 persons with T2DM patients and 45 healthy control. Venous blood samples five milliliters of sample was collected and divided into 2 parts: 2 ml in to EDTA tube for molecular study and HbA1c test, while another 3 ml put in a plain tube for collection of serum. The concentration of glucose, lipid profile, and HbA_{1c} was done by use a colorimetric methods. Determination of betatrophin was done by ELISA method in addition to the study of polymorphisms in TC7L2 gene by sequencing.

Serum level of glucose, HbA1c, cholesterol (TC) triglycerides, LDL-C, VLDL-C found to be significantly increased in patient when compared with healthy control. Whilst HDL-C and betatrophin level were found to be non-significantly changed in patients when compared with healthy control. TCF7L2 gene (rs12255372) polymorphism found to have three genotype (GG, GT, and TT) corresponding to the alleles G and T. The homozygote genotype of wild allele showed a higher frequency in control (75%) than in patients (12.5%), while the heterozygote genotype showed a higher frequency in patients (43.5%) than in control (25%) (p= 0.0001, odd ratio= 10.5 and C.I. were 14.0361 to 4864.7110). In another hand none of the control samples showed the TT genotype while patients showed (43.5%) frequency (p= 0.0002, odd ratio= 261. The TT genotype showed higher frequencies in patients than in control and it has a highly significant risk factor.

Key words: Type 2 diabetes mellitus (T2DM), TCF7L2 gene, Betatrophin level

Introduction

Type 2 diabetes mellitus (T2DM) develops because of insulin resistance of human organs and insufficient secretion of insulin from pancreatic β-cells [1]. Patients with

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T2DM may proceed with severe long-term complications including heart, kidney, retinopathy, and foot problems that increase the risk of mortality [2].This expanded his previously described "triumvirate" approach to the pathogenesis of T2DM, which identified only 3 major shortfalls: insulin resistance in the muscle, insulin resistance in the liver, and β cell failure [3].There is an interplay in the pathophysiologic dysfunction seen in T2DM among multiple organ systems throughout the body. The ominous octet involves major deficits observed in the liver, brain, muscle tissue, adipose tissue, kidneys, gastrointestinal (GI) tract, and pancreas A major shift in the epidemiology of diabetes towards those aged over 60 years has been recognized for some time. However, it is now universally recognized that all regions of the world are experiencing rapid growth in the prevalence of diabetes and this has been confirmed recently, showing that, in 2019, 488 million adults aged 20–99 years (9.5%) live with diabetes, worldwide. The epicenters of diabetes prevalence in the last decade have been China, India, the United States of America, Pakistan and Brazil In 2019, it is estimated that 19.3% of people aged 65–99 years (135.6 million, 95% CI: 107.6–170.6 million) live with diabetes. It is projected that the number of people older than 65 years (65–99 years) with diabetes will reach 195.2 million by 2030 and 276.2 million by 2045. Diabetes prevalence in people older than 65 years by International polygenic Disorder Federation (IDF) Region in 2017, 2019, 2030 and 2045. Countries with the highest number of people with diabetes (65–99 years) are China, the United States of America and India. Significant regional differences are present [4]. The metabolic acute complications are relatively short-term complications and include ketoacidosis and hyperosmolar nonketonic coma [5].

The chronic complications are conventionally categorized into two complications namely vascular complications. The vascular complications are further subcategorized into microvascular and macrovascular complications. The microvascular complications include neuropathy, nephropathy, and retinopathy, whereas, the macrovascular complications include Cerebrovascular Disease (CVA), Coronary Artery Disease (CAD) and Peripheral Vascular Disease (PVD) [6].

Modification of lifestyle, including weight loss, increasing physical activity and adopting a healthy diet, remains one of the first-line strategies for the management of T2DM [7]. The treatment of T2DM safeguards patient-centered therapeutic individualization and is initiated by the alteration of the individual lifestyle, counterworking sedentarism, and obesity through the increase of physical activity and adoption of a balanced diet [8].

Transcription factor 7 Like 2 (TCF7L2) (also known as Transcription Factor 4) Genes have important roles in the pathogenesis of T2DM is located on chromosome 10q25.2–25.3, spanning 215.9 Kb [9] and consists of 17 exons, whereas the homologous gene in mouse is located on chromosome 19 [10,11]. They also assessed the association of five Single Nucleotide Polymorphism SNPs (rs12255372, rs7903146, rs7901695, rs11196205, and rs7895340) located within the introns of the TCF7L2 gene, with T2DM [12].

Most published epidemiological studies have placed emphasis on rs7903146 (C/T) and rs12255372 (G/T) variations [13,14], with the consequential association of

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rs7903146 TCF7L2 polymorphism with T2DM [15]. TCF proteins belong to a family of transcription factors that contain high mobility group box DNA-binding domains. TCF4, encoded by TCF7L2, plays a key role in the Wnt signaling pathway it's binding by b catenin after Wnt activation of its receptor [16].

TCF7L2 exerts its regulatory effect on the Wnt signaling pathway. This pathway may play a key role in both islet cell proliferation and differentiation [17]. In humans, it had been suggested that T2DM may have a link to a mutation in the TCF7L2 gene associated with the Wnt pathway [18]. The transcription factor 7 like 2 (TCF7L2) gene is one of the most relevant risk-relate genes for T2DM [19].

Betatrophin is a newly characterized circulating hormone that is produced in tissues such as adipose tissue and liver and stimulates pancreatic beta-cell proliferation. Moreover, hepatic overexpression of betatrophin leads to increased beta-cell proliferation, islet size, insulin content, and improved glucose homeostasis [20]. Previous report showed that betatrophin-encoded protein could significantly promote the proliferation of mouse pancreatic beta cells with increasing number so as to enhance glucose tolerance [21].

Subjects and Methods

The study was conducted in Hilla-Iraq, from November 2019 to july 2020. This case-control study include 90 subjects. They were collected from Merjan Teaching Hospital in Hilla City in Babylon Province. The study was performed at the laboratory of the Biochemistry Department at the College of the Medicine, University of Babylon. 45 patients with T2DM, the ages of the patients ranged between 40-60 years. The patients was selected depend on many criteria to avoid many confounder such as without inflammation, infection that may be influence the level of betatrophin and not has any autoimmune disease. In addition to 45 persons with control subjects, with age ranged between 40-60 years. The health group have no family history of T2DM in first-degree relatives and free from inflammation or infection.

Five milliliters of blood were collected in 2 tube, one plane tube without anticoagulants for measurement the betatrophin by using ELISA kit (Bioassay) and other with EDTA tube, which was used for fresh isolation of DNA by Quick-gDNA™ Blood MiniPrep Biotechnology (Zymo/USA), rs12255372 (G/T) polymorphism in TCF7L2 gene was detected using Sequencing and Sequence Alignment. The specific Taqman probe and primer were used to detection of rs12255372 polymorphism. The characteristic of primers and probe of the TCF7L2 gene, rs 12255372 expressed in the Table 1.

Table 1 The characteristic of primers and probe of the TCF7L2 gene, rs 12255372

Primer	Sequence	Tm (°C)	GC (%)	Product size
Forward	5'-GTGGAACGTGGTTGCACAAA- 3'	59.9	60	1308bp
Reverse	5'-ACAGTTGGGAAGCATCTGGG- 3’	56.4	50	

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INtRON's Maxime PCR PreMix Kit has not only various kinds of PreMix Kit according to experience purpose, but also a 2X Master mix solution. MaximePCR Pre Mix Kit (i-Taq) is the product what is mixed every component: i-Taq DNA Polymerase, Dntpmixture, reaction buffer, and so on-in one tube for 1 rxn PCR. This is the product that can get the best result with the most convenience system. The first reason is that it has every components for PCR, so it can do PCR just add a template DNA, primer set, and distilled water. The second reason is that it has gel loading buffer to do electrophoresis, so it can do gel loading without any treatment. It is suitable for various sample's experience by fast and simple using method.

The sequenced TCF7L2 gene (rs12255372) standards (GG wild type, and GT heterozygous variant and TT homozygous variant types) were used as in NCBI where it was reference allele G and alternative allele T [22]

Results and Discussion

Serum level of glucose and HbA1c found to be significantly increased in patient when compared with healthy control at ($p<0.001$) as shown in Table 2.

Table 2 :Glucose and HbA1c levels in patients with T2DM compared to control groups

Variables	Groups	Mean	Std. Error Mean	P value
Glucose (mg/dl)	Control	6.24	0.4	0.001
	Patients	10.71	0.84	
HbA1C(mmol/mol)	Control	5.4	0.1	0.001
	Patients	8.26	0.26	

Total cholesterol (TC), triglycerides (TG), LDL-C, VLDL-C found to be significantly increased in patients when compared with healthy control at ($p<0.001$) as shown in Table 3.Whereas HDL-C increased non-significantly in control than patients, as shown in Table 3.

Table 3: Lipid profile in the study groups.

Variables	Groups	Mean	Std. Error Mean	P value
Cholesterol (mmol/L)	Control	5.7	0.24	0.001
	Patients	3.7	0.22	
Triglyceride (mmol/L)	Control	1.12	0.11	0.001
	Patients	1.81	0.1	
HDL-C (mmol/L)	Control	1.2	0.03	0.77
	Patients	1.18	0.07	
LDL-C (mmol/L)	Control	1.68	0.13	0.001
	Patients	0.59	0.08	

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VLDL-C(mmol/L)	Control	0.5	0.05	0.001
	Patients	0.82	0.04	

Serum betatrophin concentration was decreased in patients with T2DM than control groups non-significantly change at ($p>0.861$), as shown in the Table 4.

Table 4: Serum level of Beta-Trophin in patients with T2DM compared to control groups.

Variables	Groups	Mean	Std. Error Mean	P value
Beta-Trophin (ng/ml)	Control	0.32	0.04	0.681
	Patients	0.31	0.03	

Transcription Factor 7-Like 2 (TCF7L2) Gene

The sequencing of amplified product of TCF7L2 gene of study groups of Iraqi showed 100% compatibility with Homo sapiens for TCF7L2 gene, from gene bank results as shown in figure 1. Sequence ID: NG-012631.1,and number score (37.4) bits, expect 8.9% , identities 100% and gap 0%.

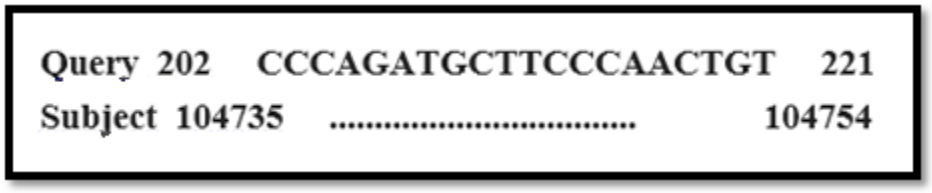

Figure 1: The sequencing of standard TCF7L2gene of Gene Bank (NCBI).

By sequencing of nucleotides of TCF7L2 gene (rs: 12255372). The graphical curve file shows the following four different genotype

1-One peak of genotyping GG figure 2. That means no change amino acids, and predicted effect nonsense.

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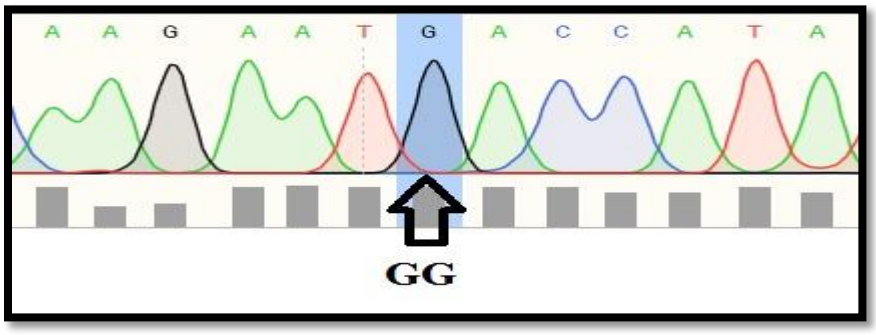

Figure 2: Photographic of Macrogen appeared GG genotyping.

2- Two peaks of genotyping GT figure 3. That means there is a change in an amino acid and predicted effect missense.

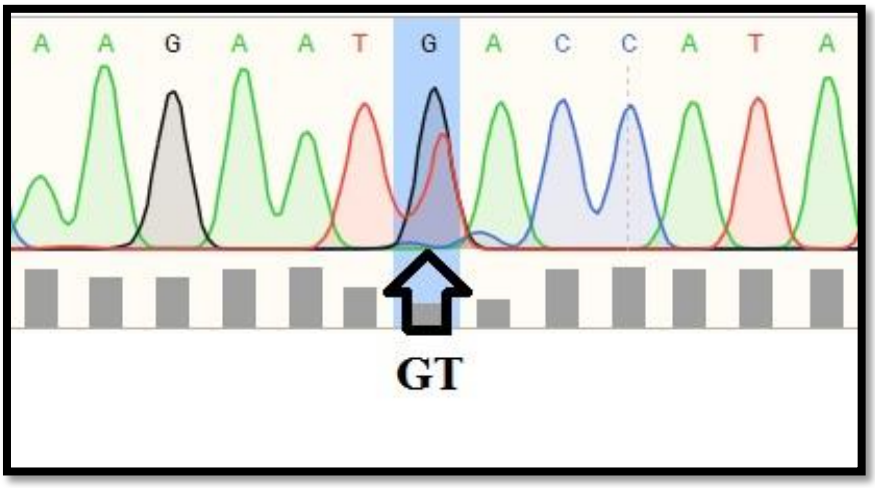

Figure 3: Photographic of Macrogen appeared GT genotyping.

Genotype Distribution and Allele Frequency

The in tronic SNP TCF7L2 gene (rs12255372) which possess three genotype (GG, GT, and TT) corresponding to the alleles G and T. the homozygote genotype of wild allele showed a higher frequency in control (75%) than in patients (12.5%), while the heterozygote genotype showed a higher frequency in patients (43.5%) than in control (25%) (p= 0.0001, odd ratio= 10.5 and C.I. were 14.0361 to 4864.7110). In another hand none of the control samples showed the TT genotype while patients showed (43.5%) frequency (p= 0.0002, odd ratio= 261 and C.I. were 14.0361 to 4864.7110). the comparison between the frequencies within the same group showed insignificant differences in both control and patients (p= 0.5882 and 0.9782, respectively). In respect to allele differences the G allele were significantly higher in control (91%) than in patients (33%) while the T allele were higher in patients (63%) than in control (13%), as shown in Table 4.

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Table 4: Genotype Frequency GG, GT and TT of study groups.

Genotype Frequency (%) rs12255372					
Genotype	**Control n = 52**	**Cases n = 48**	**P-value**	**Odds Ratio**	**95% Cl**
GG	39 (75%)	6 (12.5%)	----	1.0	----
GT	13 (25%)	21 (43.5%)	< 0.0001**	10.5	3.4837 to 31.6471
TT	0 (0%)	21 (43.5%)	0.0002**	261.3	14.0361 to 48.12
Chi-square	1.0612 NS	0.0441 NS			
P Value	0.5882	0.9782			
Allele frequency (%)					
Allele	**Control n=104**	**Cases n=96**	**P-value**	**Odds Ratio**	**95% Cl**
G	0.875 (91)	0.34 (33)	<0.0001**	13.36	6.494 to 26.23
T	0.125 (13)	0.66 (63)			

The results of Hardy frequencies distribution of genotypes showed a good agreement with Weinberg equation as summarized in Table 5 in both control and patients (0.59 and 0.98, respectively).

Table 5: The percentage frequencies of genotype and alleles for TCF7L2 gene (rs12255372) and Hardy-Weinberg Equilibrium (HWE).

			Genotypes			**HWE p>0.05 (X^2)**	**Alleles**	
			Wild GG	**Hetero GT**	**Variant TT**		**G reference allele**	**T variant allele**
Control (N=52)	**Observed**	**No.**	**39**	**13**	**0**	**0.59 (0.044)**	**91**	**13**
		%	**75**	**25**	**0**		**87.5**	**12.5**
	Expected	**No.**	**39.8**	**11.4**	**0.8**			
		%	**75.5**	**21.9**	**1.5**			
Cases (N=48)	**Observed**	**No.**	**6**	**21**	**21**	**0.98 (1.061)**	**33**	**63**
		%	**12.5**	**43.5**	**43.5**		**34.4**	**65.63**
	Expected	**No.**	**5.7**	**21.7**	**20.7**			
		%	**11.9**	**45.21**	**43.13**			

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The genetic model of the association for alleles and genotypes of TCF7L2 gene (rs12255372) in co-dominant, dominant, recessive, and overdominant models.

Scanning of TCF7L2 gene

Scanning of TCF7L2 gene (rs:12255372) reveals for different groups according to the presence or absence of nitrogenous base substitution, which are:

After alignment of product amplification of TCF7L2 gene with standard TCF7L2 gene in Gene Bank of second group appears with two Transition G/A,and C/T in locations (103867,103959 nucleotide), and four Transvertion three G\C in locations (103822,103839 and 103876nucleotide), and one G/T in location (103894 nucleotide) that have 99% compatibility with the standard TCF7L2 gene as figure 4 shows. This sequence was submitted by gene bank NCBI, DNA data bank of Korea/Macrogen.

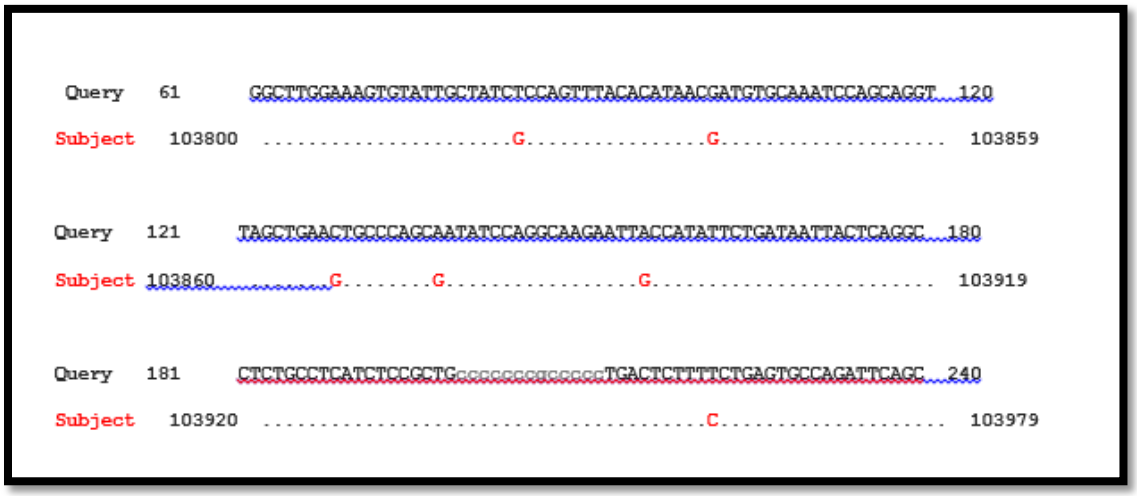

Figure 4: Compatibility with the standard TCF7L2 gene submitted by gene bank NCBI, DNA data bank of Korea/Macrogen.

The sequenced TCF7L2 gene(**rs12255372**) standards (GG wild type, and GT heterozygous variant and TT homozygous variant types) were used as in NCBI where it was reference allele G and alternative allele T.

The relationship between beta-trophin and biochemical markers in the current study using Pearson correlation analysis. The collective results are presented in Tables 5. In the present study Beta Trophin level showed a significant positive relation with HDL-C (r=0.29, 95% CI= 0.004 -0.54,p=0.04).

	r value	95% CI	Sig	P value
Glucose	**-0.14**	**-0.41 to 0.15**	**NS**	**0.35**
Cholesterol	**0.10**	**-0.19 to 0.38**	**NS**	**0.51**
Triglyceride	**0.03**	**-0.26 to 0.32**	**NS**	**0.83**

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HDL	**0.29**	**0.004 to 0.54**	*****	**0.04**
HbA1c	**0.01**	**-0.28 to 0.30**	**NS**	**0.94**
LDL	**-0.09**	**-0.38 to 0.20**	**NS**	**0.51**
vLDL	**0.03**	**-0.26 to 0.32**	**NS**	**0.84**

Table 5: Correlations between beta-trophin in patients with T2DM and biochemical markers compared to control groups.

Conclusions

The TT genotype of TCF7L2 gene showed higher frequencies in patients with T2DM than in control and it has a highly significant risk factor and may play a critical role in the pathogenesis of T2DM.

Present study shows no correlation between serum concentration of beta-trophin and pathogenesis of T2DM in Iraqi patients.

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