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Original Scientific Paper

EXPRESSION PROFILE OF *ADIPOQ* AND *TCF7L2* GENES IN TYPE 2 DIABETIC PAKISTANI SUBJECTS

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SUMMARY – The purpose of this study was to investigate the expression of the *ADIPOQ* and transcription factor 7-like 2 (*TCF7L2*) genes in type 2 diabetes mellitus (T2D) in a Pakistani population. This aim of the study was to find the potential link between the expression of the *ADIPOQ* and *TCF7L2* genes in T2D. This cross-sectional study included 200 patients with T2D and 200 control subjects. Analysis of the expression of the *ADIPOQ* and *TCF7L2* genes was performed by real-time polymerase chain reaction. A significant increase in the expression of *ADIPOQ* and *TCF7L2* genes was observed in T2D group as compared to control group. In T2D group, a significant positive relationship was found between the expression of the *ADIPOQ* gene and systolic blood pressure, random blood glucose and expression of *TCF7L2* gene. A significant negative correlation was observed between the expression of the *TCF7L2* gene and diastolic blood pressure. Multivariable linear regression analysis revealed that the expression of *TCF7L2* gene (β=0.274; adjusted R^2 change, 0.135; p=0.005) and expression of *ADIPOQ* gene (β=0.270; adjusted R^2 change, 0.148; p=0.001) were positively associated in T2D, whereas diastolic blood pressure (β=-0.296; adjusted R^2 change, 0.148; p=0.001) was negatively associated in T2D as compared to control group. When stepwise linear regression was performed considering the expression of the *TCF7L2* gene as a dependent variable in T2D group, one model was computed showing the expression of *ADIPOQ* gene (β=0.335, p=0.001) as an important predictor of T2D. When stepwise linear regression was employed considering the expression of *ADIPOQ* gene as a dependent variable, two models were computed showing the expression of *TCF7L2* gene (β=0.335; p=0.001) and systolic blood pressure (β=0.138; p=0.041) as important determinators of T2D. Thus, the expression of the *ADIPOQ* and *TCF7L2* genes are independent predictors of T2D. In conclusion, there was increased expression of the *ADIPOQ* gene and *TCF7L2* gene in T2D subjects as compared to controls. The *ADIPOQ* gene expression and *TCF7L2* gene expression provide independent predictors of T2D indicating their role in the development of T2D. Future research is required to elucidate the mechanisms involved in the increased *ADIPOQ* and *TCF7L2* gene expression in T2D.

Keywords: *Type 2 diabetes; ADIPOQ gene; TCF7L2 gene; Gene expression; Pakistani population*

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Introduction

The incidence of type 2 diabetes mellitus (T2D), a metabolic condition with serious consequences, is on the rise globally, presenting a significant public health challenge. According to the International Diabetes Federation (IDF), around five hundred million people worldwide have received a diabetes diagnosis. Projections suggest an increase to 693 million by 2045[1]. It has been suggested that a confluence of hereditary and environmental factors contributes to the development of resistance to insulin in T2D[2]. Several environmental factors have been recognized as precursors of T2D, including central obesity, low physical activity, a diet high in fat, hypertension, and reduced glucose tolerance[2,3]. In addition, genetics may also significantly contribute to the occurrence of T2D across various populations. The chance of having T2D is higher in those who have a genetic susceptibility[2].

Adiponectin is a protein hormone and adipokine that regulates glucose levels and breakdown of fatty acids. It is also known by the names GBP-28, apM1, AdipoQ, and Acrp30. The *ADIPOQ* gene encodes it, and in humans, adipose tissue, muscle, and even the brain are the main places where it is generated. The *ADIPOQ* gene which is situated on chromosome 3q27 and encodes adiponectin, has been suggested as a genomic locus associated with T2D based on genome-wide scans. Additionally, studies have indicated that individuals with T2D also exhibit lower levels of adiponectin in circulation[4]. It is understood that adiponectin possesses properties that are anti-atherosclerotic, anti-inflammatory, and anti-diabetic. Research has revealed that individuals with metabolic syndrome, insulin resistance, and T2D exhibit reduced levels of adiponectin, which is a protein (~30 kDa) secreted by adipose tissue[4].

Human *TCF7L2* gene was first sequenced in colorectal cancer cell lines and found to be located on chromosome 10q25.3[5,6]. Transcription factor *TCF7L2*, also known as TCF4, is member of the T-cell factor/lymphoid enhancer binding factor (TCF/LEF) family and binds to DNA using a high-mobility group domain. This gene has gained significant interest due to its robust genetic link with T2D[7,8]. The Finnish Diabetes Prevention Study conducted in 2007 revealed that certain *TCF7L2* variations were capable of predicting the onset of T2D and were linked to compromised glucose regulation and insulin secretion[9]. Insulin secretion mediated by glucose is negatively impacted by TCF7L2 silencing. The fusion of insulin-secretory granules has been proposed as the possible source of the problem. Therefore, the incidence of T2D among bearers of at-risk TCF7L2 alleles might be explained by defective insulin exocytosis[10]. Studies on *in vitro* overexpression of gluconeogenic precursors using chromatin immunoprecipitation in conjunction with massive DNA sequencing (ChIP-Seq) across the genome and TCF7L2 silencing demonstrated that TCF7L2 was a crucial regulator of hepatic glucose metabolism; consequently, risk variants may impact postprandial glycemia, as well as fasting glycemia in carriers of T2D risk genotypes[11].

It is well known that adipocyte development is inhibited by Wnt-signaling pathway activation. TCF transcription factors, such as *TCF7L2* (transcription factor-7-like 2), are the primary intracellular effectors of the Wnt-signaling pathway. Through genome-wide association studies in different human groups, several genetic variations close to *TCF7L2* have been associated with T2D. *TCF7L2* controls the growth of adipocytes, insulin secretion, and glucose metabolism. In models of obesity produced by food or genetics, *TCF7L2* expression was decreased. *TCF7L2* is abundantly expressed in white adipose tissue. *TCF7L2* directly controls genes involved in cellular metabolism and cell cycle regulation, according to a study of gene expression in adipocytes and genome-wide distribution of *TCF7L2* binding. Conditional loss of *TCF7L2* in adipocytes induced increased adipose tissue mass, reduced insulin sensitivity, impaired glucose tolerance, and promoted weight gain when exposed to a high-fat diet. This was associated with decreased triglyceride hydrolase expression, decreased free fatty acid release produced by fasting, and decreased adipocyte hypertrophy in subcutaneous adipose tissue[12].

Adiponectin gene and *TCF7L2* are situated on chromosomes 10q25.3 and 3q27, respectively, and three diabetes susceptibility loci have been discovered by recent genome-wide scans. Taiwanese T2D patient adiponectin and *TCF7L2* gene polymorphisms were investigated. Taiwanese patients with T2D seldom have *TCF7L2* polymorphisms. T2D patients with TT

genotype on the adiponectin gene SNP-45 exhibited lower levels of high-density lipoprotein cholesterol than patients with TG and GG genotypes. The levels were lower in women with GG genotype than in men with GG genotype[13]. In our population, the expression profile of *ADIPOQ* and *TCF7L2* genes has not been reported before. So, this study aimed to investigate the expression of the *ADIPOQ* and *TCF7L2* genes in T2D in the Pakistani population. We also aimed to find the potential link between the expression of *ADIPOQ* and *TCF7L2* genes in T2D patients.

Material and Methods

In this cross-sectional study, subjects with T2D who attended Department of Endocrinology and Metabolism, Services Institute of Medical Sciences in Lahore from May to June 2022 were enrolled. This research was permitted by the Ethics Review Committee, Lahore College for Women University (Zoo/LCWU/467) and Services Institute of Medical Sciences (Ref No. IRB/2022/957/SIMS from March 24, 2022), Lahore. A total of 400 samples were selected out of which 200 were T2D patients and 200 subjects did not have any disease and were considered a control group. Control group samples were collected from a random population. All subjects were recruited after receiving their informed consent for participation in this study.

Subjects who had gestational diabetes or neonatal diabetes mellitus, diabetic patients suffering from other severe illnesses such as renal impairment, anemia, cardiovascular diseases including recent stroke, transient ischemic attack and coronary stent placement within six months, hemorrhage in the past six months, hepatitis and cancer were excluded.

Questionnaire and consent form

Data on the study population were collected by using a self-designed questionnaire. It included demographic characteristics such as age, gender, body weight, body height, blood pressure, random blood glucose (RBG) level, body mass index (BMI), prevailing symptoms, risk factors, lifestyle, and comorbidities. A consent form was signed by each subject before giving a blood sample and filling out the questionnaire.

Blood specimen collection and storage

A total of 5 mL of blood was collected from each subject enrolled in the study. Samples were collected in ethylenediamine tetraacetic acid (EDTA) vials to prevent blood clotting[14]. The vials were labelled with the subject identification numbers and transported to the laboratory at Lahore College for Women University. Serum was separated and RNA extraction was performed, stored at a -40 ˚C till further assessment.

Blood pressure and BMI measurements

The sphygmomanometer was used to measure both systolic blood pressure (SBP) and diastolic blood pressure (DBP). Medically trained staff of the hospital supervised the process. BMI of both groups was calculated.

Biochemical assessments

Fasting blood glucose (FBG) and RBG were measured with a glucometer. Serum uric acid (SUA) and glycated hemoglobin (HbA1c) were measured on a chemistry analyzer (ERBA Chemistry Analyzer, Model# CHEM-7, Serial# 9047; Erba Diagnostics Mannheim GmbH, Germany) using biochemical kits at the research laboratory of the Zoology Department, Lahore College for Women University.

RNA isolation

Within 2-4 hours of blood collection, the samples were processed for total RNA extraction using TRIzol reagent. During this process, a blood sample of 500 µL was taken in a 1.5 mL eppendorf tube and 700 µL of TRIzol reagent was added to it. The mixture was gently shaken and incubated at room temperature for 5 minutes. Subsequently, 200 µL of chloroform was added to the sample, which was then shaken well for 15 seconds, incubated at room temperature for 2 minutes, and shaken again for 1 minute. The mixture was then kept at room temperature for 10 minutes. It was centrifuged at 12,000 rpm and 4 ˚C for 15 minutes, resulting in the formation of two layers and an interphase, with the RNA present in the upper aqueous layer. This upper layer was transferred to a new 1.5 mL eppendorf tube, to which 500 µL of isopropanol was added. The mixture was gently overturned and incubated at room temperature for 10 minutes, followed by centrifugation at 12,000 rpm and 4 ˚C for 10 minutes, leading

to the formation of a white RNA pellet at the bottom of the tube. The supernatant was discarded, and the RNA pellet was washed with 500 µL of 75% ethanol by gently overturning the tube. After incubating for 3 minutes at room temperature, ethanol was discarded, and this washing step was repeated. The tube was then overturned on a clean permeable paper to completely remove 75% ethanol, followed by air drying for 10 minutes. Finally, 30 µL of RNAase-free water was added to the eppendorf tube, which was incubated at room temperature for 10 minutes and then stored at -80 °C. A Nanodrop spectrophotometer (Multiskan SkyHigh Microplate Spectrophotometer, UK) was used to determine the concentration and purity of the extracted RNA. It calculates the concentration of total RNA in a sample by measuring absorbance at 260 nm.

cDNA synthesis

Only mRNA is reverse transcribed into cDNA during the cDNA synthesis process, with the help of the Maxima® First Stand cDNA Synthesis Kit (Thermo Scientific RevertAid First Stand cDNA Synthesis Kit, cat# K1622; Thermo Fisher Scientific, USA) because oligo(dT) primers attach to the poly-A tail of mRNA. Other RNA types, such as tRNA and rRNA, stay in the solution and are not reverse transcribed into cDNA. They could not substantially obstruct the synthesis of cDNA from mRNA, although they can exist as contaminants in the RNA sample. Gel electrophoresis was performed to confirm cDNA.

Expression analysis of real-time polymerase chain reaction

Oligonucleotide primers were designed through Primer 3 software for the process of real-time polymerase chain reaction (RT-PCR) (Applied Biosystems Step OneTM Real-Time PCR system, Thermo Scientific Fisher Inc., USA). The primer sequences and their optimization conditions are shown in Table 1. The primers were generated by an easily accessible commercial industry (MACROGEN, 238, Teheran-ro, Gangnam-gu, Seoul, Republic of Korea). Relative expression of *ADIPOQ* and *TCF7L2* genes was estimated using the Thermo Scientific Maxima SYBER Green/ROX qPCR Master Mix (Cat# k0221). To normalize expression of the target gene, the Glyceraldehyde 3-phosphate dehydrogenase (*GAPDH*) gene was employed as a reference. The RT-PCR procedure consisted of one cycle of 94 °C for 4 minutes, followed by 30 cycles of 94 °C for 30 seconds, 59 °C for 20-30 seconds, and 72 °C for 45 seconds. Final extension took place at 72 °C for 5 minutes.

Statistical analysis

Data were analyzed by SPSS version 22.0 software. Categorical data were expressed as frequency and percentage and continuous data as mean ± standard deviation (SD). Data were checked for normality followed by Kolmogorov-Smirnov and Shapiro-Wilk tests. Mean values of various parameters were compared by Student's t-test. Bivariate Pearson's correlation was applied to find the link of the expression of the *ADIPOQ* and *TCF7L2* genes with clinical parameters of diabetes such as age, SBP, DBP, BMI, FBG, RBG, HbA1c and uric acid in study subjects. The impact of the *ADIPOQ* gene and *TCF7L2* gene expression on clinical parameters in the T2D group was investigated using linear and stepwise regression analysis. Data on gene expression were expressed as a fold change, and comparative CT (2-ΔΔCT) was used to calculate relative gene expression levels. The level of statistical significance was set at $p<0.05$.

Table 1. Primer sequences used in study groups

Gene	Primer sequence	Product size (bp)	TM (°C)
GAPDH	F: ATCCCATCACCATCTTCCAGGA R: CAAATGAGCCCCAGCCTTCT	122	59
ADIPOQ	F: CATGACCAGGAAACCACGACT R: TGAATGCTGAGCGGTAT	301	59
TCF7L2	F: AAAGCGCGGCCATCAAC R: CAGCTCGTAGTATTTCGCTTGCT	528	59

TM = temperature

Results

The gender, thirst status, frequent urination, hunger status, hypertension and smoking status in the study groups are shown in Table 2. The mean ± SD values of variables such as age, SBP, DBP, BMI, FBG, RBG, HbA1c, and SUA in control group and T2D group are shown in Table 2. Statistically significant differences in SBP, DBP, BMI, FBG, RBG, HbA1c and SUA were noted between the study groups (Table 2).

Assessment of ADIPOQ gene and TCF7L2 gene expression

The n-fold difference with the reference gene (*GAPDH*) was used to quantitatively express relative expression of the genes. A highly significant difference between the groups was found in the expression profile of the *ADIPOQ* gene, which increased ~10.07-fold in the T2D group and ~1.32-fold in the control group (Table 2), whereas the expression profile of the *TCF7L2* gene increased ~13.81-fold in the T2D group and ~1.57-fold in the control group, yielding a highly significant difference between the groups (Table 2).

Pearson's correlation analysis

Pearson's correlation analysis was performed to assess the relationship of the expression of the *ADIPOQ* and *TCF7L2* genes with clinical parameters of T2D such as age, SBP, DBP, BMI, FBG, RBG, HbA1c and SUA. A significant negative correlation between the expression of *ADIPOQ* gene and *TCF7L2* gene (r=0.154, p=0.029) and SBP (r=-0.267, p=0.001), and

Table 2. Baseline demographics, risk factors, comorbidities and clinical parameters in study groups

Clinical parameter		Control group (N=200)	T2D group (N=200)	T-test p-value
Age (years)		50.70±6.87	50.50±8.05	0.789
Male (n %) Female (n %)		120 (60%) 80 (40%)	96 (48%) 104 (52%)	NA
Increased thirst (n, %)	Yes No	0 (%) 200 (100%)	108 (54%) 92 (46%)	NA
Frequent urination (n, %)	Yes No	0 (%) 200 (100%)	124 (62%) 76 (38%)	NA
Extreme hunger (n, %)	Yes No	0 (%) 200 (100%)	72 (36%) 128 (64%)	NA
Hypertension (n, %)	Yes No	0 (%) 200 (100%)	88 (44%) 112 (56%)	NA
Smoking (n, %)	Yes No	0 (%) 200 (100%)	72 (36%) 128 (64%)	NA
SBP (mmHg)		126.28±10.90	139.72±20.88	0.001
DBP (mmHg)		80.56±8.73	86.16±10.92	0.001
BMI (kg/m^2)		22.98±1.90	27.28±4.53	0.001
FBG (mg/dL)		110.73±21.00	265.63±89.06	0.001
RBG (mg/dL)		134.80±16.64	268.02±83.06	0.001
HbA1c (%)		4.74±0.73	9.45±1.61	0.001
SUA (mg/dL)		6.08±1.96	7.41±3.37	0.001
Expression of *ADIPOQ* gene (fold change)		1.32±1.04	10.07±9.60	0.001
Expression of *TCF7L2* gene (fold change)		1.57±1.41	13.81±10.86	0.001

Categorical data were expressed as frequency and percentage and continuous data as mean ± SD; NA = not applicable; SBP = systolic blood pressure; DBP = diastolic blood pressure; BMI = body mass index; FBG = fasting blood glucose; RBG = random blood glucose; SUA = serum uric acid; HbA1c = glycated hemoglobin

positive association with age (r=0.145, p=0.040) was observed in the control group (Table 3). An inverse significant relationship was found of the expression of *TCF7L2* gene with SUA (r=-0.235, p=0.001), and the expression of *ADIPOQ* gene (r=-0.154, p=0.029) in the control group (Table 3).

In the T2D group, a significant positive relationship was found between the expression of *ADIPOQ* gene and SBP (r=0.176, p=0.013), RBG (r=0.156, p=0.027) and expression of *TCF7L2* gene (r=0.335, p=0.001) (Table 3). Also, a significant positive correlation was found between the expression of *TCF7L2* gene and expression of *ADIPOQ* gene (r=0.335, p=0.001) but negative correlation with DBP (r=-0.145, p=0.041) (Table 3).

Linear regression analysis

Tables 4 and 5 show linear regression analysis in control and T2D groups. In multivariable linear regression analyses, the expression of *TCF7L2* gene (β=0.274; adjusted R^2 change, 0.135; p=0.005) and expression of *ADIPOQ* gene (β=0.270; adjusted R^2 change, 0.148; p=0.001) were positively associated in T2D group, whereas DBP (β=-0.296; adjusted R^2 change, 0.148; p=0.001) was negatively associated in T2D group.

Stepwise linear regression analysis

When stepwise linear regression was applied, beta coefficient allowed direct comparisons between independent variables to determine which influenced the dependent variable most. The independent variable is a condition that acts on the dependent variable. The dependent variable is a condition that researchers measure to understand the degree to which an independent variable causes an effect. A stepwise linear regression was carried out in the study groups keeping the expression of the *ADIPOQ* and *TCF7L2* genes as dependent variables, and age, SBP, DBP, BMI, FBG, RBG, HbA1c, and SUA as independent variables because they are most reliable risk factors of T2D. That is why we selected these clinical parameters.

In control group, stepwise linear regression was applied using expression of the *TCF7L2* gene as a dependent variable, whereas age, SBP, DBP, BMI, FBG, RBG, HbA1c, and SUA were considered independent variables. Two models were calculated displaying SUA (β=-0.235; p=0.001) and expression of the *ADIPOQ* gene (β=-0.136; p=0.049). When stepwise linear regression was applied using expression of the *ADIPOQ* gene as a dependent variable, 3 models were computed showing SBP (β=-0.267; p=0.001), age (β=0.185; p=0 .007) and DBP (β=0.186; p=0.007) in control group (Table 6).

Table 3. Correlation analysis of ADIPOQ gene and TCF7L2 gene expression with T2D clinical parameters

Clinical parameter	r-value of *ADIPOQ* gene expression		r-value of *TCF7L2* gene expression	
	Control group	T2D group	Control group	T2D group
Age (years)	0.145*	0.127	-0.058	0.114
SBP (mmHg)	-0.267**	0.176*	0.068	0.121
DBP (mmHg)	0.106	-0.054	-0.086	-0.145*
BMI (kg/m²)	0.008	-0.051	0.080	-0.025
FBG (mg/dL)	0.041	-0.051	-0.015	0.029
RBG (mg/dL)	0.008	0.156*	-0.005	0.070
HbA1c (%)	0.032	-0.033	0.021	-0.094
SUA (mg/dL)	0.081	0.099	-0.235*	0.035
Expression of *ADIPOQ* gene (fold change)	-----	-----	-0.154*	0.335*
Expression of *TCF7L2* gene (fold change)	-0.154*	0.335*	-----	------

**Correlation significant at the 0.01 level (2-tailed); *correlation significant at the 0.05 level (2-tailed);
SBP = systolic blood pressure; DBP = diastolic blood pressure; BMI = body mass index; FBG = fasting blood glucose; RBG = random blood glucose; SUA = serum uric acid; HbA1c = glycated hemoglobin

Table 4. Linear regression analysis in control group

Variable	Control group			
	Expression of *TCF7L2* gene		Expression of *ADIPOQ* gene	
	Model 1		Model 1	
	Beta	p-value	Beta	p-value
(Constant)	----	0.446	---	0.527
Age (years)	-0.052	0.504	0.162	0.031
SBP (mmHg)	0.063	0.430	-0.343	0.001
DBP (mmHg)	-0.064	0.392	0.170	0.016
BMI (kg/m^2)	0.068	0.401	0.005	0.950
FBG (mg/dL)	0.003	0.968	0.045	0.505
RBG (mg/dL)	-0.012	0.873	0.042	0.559
HbA1c (%)	-0.019	0.787	0.054	0.427
SUA (mg/dL)	-0.212	0.004	0.041	0.560
Expression of *ADIPOQ* gene (fold change)	-0.106	0.162	----------	--------
Expression of *TCF7L2* gene (fold change)	----------	----------	-0.097	0.162

SBP = systolic blood pressure; DBP = diastolic blood pressure; BMI = body mass index; FBG = fasting blood glucose; RBG = random blood glucose; SUA = serum uric acid; HbA1c = glycated hemoglobin

Table 5. Linear regression analysis in T2D group

Variable	Control group			
	Expression of *TCF7L2* gene		Expression of *ADIPOQ* gene	
	Model 1		Model 1	
	Beta	p-value	Beta	p-value
(Constant)	---	0.116	---	0.261
Age (years)	0.041	0.626	0.089	0.288
SBP (mmHg)	0.225	0.017	0.177	0.063
DBP (mmHg)	-0.296	0.001	-0.121	0.192
BMI (kg/m^2)	0.060	0.416	0.005	0.942
FBG (mg/dL)	0.140	0.068	-0.046	0.558
RBG (mg/dL)	0.064	0.400	0.147	0.054
HbA1c (%)	-0.154	0.044	0.001	0.985
SUA (mg/dL)	-0.007	0.915	0.082	0.222
Expression of *ADIPOQ* gene (fold change)	0.270	0.001	----------	--------
Expression of *TCF7L2* gene (fold change)	----------	----------	0.274	0.001

SBP = systolic blood pressure; DBP = diastolic blood pressure; BMI = body mass index; FBG = fasting blood glucose; RBG = random blood glucose; SUA = serum uric acid; HbA1c = glycated hemoglobin

Table 6. Stepwise linear regression in control group

Variable	B	95% CI		SE B	β	p-value	R^2	ΔR^2	Sig. fold change
		LL	UL						
(Expression of *TCF7L2* gene)									
Model 1							0.055	0.055	0.001
(Constant)	3.332	2.263	4.402	0.542		0.001			
SUA	-0.289	-0.456	-0.122	0.085	-0.235	0.001			
Model 2							0.074	0.018	0.049
(Constant)	3.462	2.393	4.532	0.542		0.001			
SUA	-0.275	-0.442	-0.109	0.084	-0.224	0.001			
Expression of *ADIPOQ* gene	-0.160	-0.320	-0.001	0.081	-0.136	0.049			
(Expression of *ADIPOQ* gene)									
Model 1							0.071	0.071	0.001
(Constant)	7.651	4.436	10.866	1.630		0.001			
SBP	-0.050	-0.075	-0.025	0.013	-0.267	0.001			
Model 2							0.105	0.034	0.007
(Constant)	5.452	1.910	8.994	1.796		0.003			
SBP	-0.055	-0.080	-0.030	0.013	-0.292	0.001			
Age	0.055	0.015	0.095	0.020	0.185	0.007			
Model 3							0.137	0.032	0.007
(Constant)	3.072	-0.822	6.965	1.974		0.121			
SBP	-0.064	-0.089	-0.038	0.013	-0.340	0.001			
Age	0.055	0.016	0.095	0.020	0.185	0.006			
DBP	0.044	0.012	0.075	0.016	0.186	0.007			

CI = confidence interval; LL = lower limit; UL = upper limit; SE B = standard error beta; SUA = serum uric acid; SBP = systolic blood pressure; DBP = diastolic blood pressure

Table 7. Stepwise linear regression in T2D group

Variable	B	95% CI		SE B	β	p-value	R^2	ΔR^2	Sig. fold change
		LL	UL						
(Expression of *TCF7L2* gene)									
Model 1							0.112	0.112	0.001
(Constant)	11.948	10.337	13.559	0.817		0.001			
Expression of *ADIPOQ* gene	0.186	0.112	0.259	0.037	0.335	0.001			
(Expression of *ADIPOQ* gene)									
Model 1							0.112	0.112	0.001
(Constant)	1.726	-2.458	5.910	2.122		0.417			
Expression of *TCF7L2* gene	0.604	0.366	0.842	0.121	0.335	0.001			
Model 2							0.131	0.019	0.041
(Constant)	-15.905	-33.320	1.510	8.831		0.073			
Expression of *TCF7L2* gene	0.574	0.336	0.812	0.121	0.318	0.001			
SBP	0.129	0.005	0.253	0.063	0.138	0.041			

CI = confidence interval; LL = lower limit; UL = upper limit; SE B = standard error beta; SBP = systolic blood pressure

In T2D group, expression of the *TCF7L2* gene was considered as a dependent variable, whereas age, SBP, DBP, BMI, FBG, RBG, HbA1c, and SUA were considered as independent variables. One model was computed showing expression of the *ADIPOQ* gene (β=0.335; p=0.001) as an important predictor of T2D. When stepwise multiple regression was employed considering expression of the *ADIPOQ* gene as a dependent variable, 2 models were computed showing expression of *TCF7L2* gene (β=0.335; p=0.001) and SBP (β=0.138; p=0.041) as important determinators of T2D (Table 7).

Discussion

This study was intended to investigate expression of the *ADIPOQ* gene and *TCF7L2* gene in the Pakistani T2D population. As a result, we found increased expression of the *ADIPOQ* gene and *TCF7L2* gene in T2D group as compared to control group. A significant positive relationship was found between expression of the *ADIPOQ* gene and SBP and RBG in T2D group, whereas a significant negative correlation was found between expression of the *TCF7L2* gene and DBP. This study is the first to demonstrate a significant correlation between expression of the *ADIPOQ* gene and TCF7L2 gene in the Pakistani T2D population.

Results of our study indicated a significant positive relationship between expressions of the *ADIPOQ* gene and SBP in T2D group. Also, a significant negative correlation was observed between expression of the *TCF7L2* gene and DBP. However, to fully comprehend the negative link between expression of the *TCF7L2* gene and DBP in T2D patients, further research is required. The relationship between hypertension and T2D is bidirectional, with hypertension affecting T2D and *vice versa*. Elevated blood pressure has been shown to increase the levels of inflammatory markers associated with the insulin signalling pathway and function of beta cells, potentially accelerating the onset of diabetes[15].

A significant positive relationship was found between expression of the *ADIPOQ* gene and FBG in T2D group. The FBG and glucagon levels are not directly related. However, in individuals with T2D and glucose intolerance, there was dysregulation of glucagon suppression after glucose intake. Additionally, there seems to be a relationship between the level of glucagon and central obesity in patients with T2D[16]. An RBG value of 100 mg/dL or higher was a more significant indicator of undiagnosed diabetes compared to conventional risk variables. Therefore, aberrant RBG values should be considered as a risk factor for diabetes and included in screening recommendations[17].

In the present study, higher SUA levels were found in T2D group than in control group. In T2D patients, obesity seems to be a major factor in determining SUA levels. Diabetes may directly impact purine nucleotide oxidation, resulting in elevated SUA levels. Additionally, by accelerating the production of xanthine oxidase, hyperinsulinemia may cause hyperuricemia. The substantial correlation between T2D and obesity raises the possibility that elevated SUA levels are involved[18-20].

We found increased expression of the *ADIPOQ* gene in T2D group as compared to control group. In T2D group, when stepwise linear regression was employed considering expression of adiponectin gene as a dependent variable, 2 models were computed showing expression of *TCF7L2* gene and SBP to be important determinators of T2D. Adiponectin has various functions in coordinating metabolism. This hormone, which is secreted by adipocytes, activates two adiponectin receptors, i.e., ADIPOR1 and ADIPOR2, as well as PPARγ, leading to increase in beta-oxidation, a key pathway in the metabolism of lipids. ADIPOR1 enhances the activity of several genes[21], while also lowering the levels of important inflammatory genes. ADIPOR1 also triggers activation of p38 mitogen-activated protein kinase (p38MAPK), which is involved in transcriptional regulation. Additionally, ADIPOR1 indirectly affects the activity of endothelial nitric oxide synthase (eNOS), which controls oxidative stress, by acting on HSP90 through PI3K. Nitric oxide (NO) and eNOS production rise as a result of AMP-activated protein kinase 1 (AMPK1) being upregulated by ADIPOR2 activating APPL1 (adaptor protein, phosphotyrosine interacting with PH domain and leucine zipper 1)[21,22]. Higher AMPK also enhances the function of phosphoenolpyruvate carboxykinase (PEPCK), causing an increase in gluconeogenesis. APPL1 also acts on Akt (serine/threonine kinase),

which promotes translocation of glucose transporter type 4 and increases glucose uptake by the cell[23]. Adiponectin, being derived from adipocytes, comes into contact with blood plasma and directly affects the Adipo R1/R2 receptors, leading to the activation/inhibition of downstream genes related to oxidative stress and inflammation. Elevated protein carbonyl content, indicating increased oxidative stress, is present in the plasma and hemolysate of T2D patients[24].

Adiponectin has the potential to be a gene that contributes to susceptibility to T2D and could play a role in the development of T2D by regulating glucose and lipid metabolism in the body[25]. However, more experiments are needed to confirm the findings of this study and to gain a more complete understanding of the possible mechanisms of adiponectin in different stages of T2D. This could lead to the development of new directions for the treatment of T2D in the future[25]. The research indicates that there is a notable and significant link between the TT genotype of the SNP+276 G>T within the adiponectin gene and a higher risk of developing T2D in the European population[26]. In individuals with T2D, the mechanism behind the increased gene expression of adiponectin is not entirely clear. However, some research suggests that it may be due to the activation of certain signaling pathways in response to insulin resistance and hyperglycemia[4]. However, more research is needed to fully understand the mechanisms behind the increased gene expression of adiponectin in T2D.

We found increased expression of the *TCF7L2* gene in T2D group than in control group. Another study revealed that pancreatic islets from human cadavers exposed to T2D had a 5-fold increase in *TCF7L2* expression. More study is required, but there is some evidence that the risk genotype may be linked to higher *TCF7L2* expression in non-diabetic islets. Adenovirus-mediated overexpression of *TCF7L2* in human islets decreased insulin secretion. However, insulin gene mRNA levels varied. According to the study, *TCF7L2* may be crucial in controlling hepatic metabolism of glucose, and establishing therapies to alter its expression may aid in the treatment of T2D[27].

TCF7L2 is responsible for regulating the preproglucagon gene that encodes glucagon-like peptide 1 (GLP-1), and it has been hypothesized that *TCF7L2* variation that alters the levels of GLP-1 in endocrine cells may affect the risk of developing T2D[28]. When *TCF7L2* is silenced, it significantly impairs insulin secretion stimulated by glucose, possibly due to impaired fusion of insulin-secretory granules. This defect in insulin exocytosis could be a contributor to the elevated risk of T2D in individuals carrying the at-risk *TCF7L2* alleles[10]. *TCF7L2* is a crucial regulator of the hepatic metabolism of glucose using *TCF7L2* silencing and *in vitro* overexpression investigations of gluconeogenic precursors in conjunction with chromatin immune precipitation of chromatin and DNA sequencing. Therefore, in individuals with T2D risk genotypes, risk variations of *TCF7L2* may affect both fasting and random glycemia. Conducting functional and physiological studies on the recently identified variants is crucial to understand the molecular pathways related to T2D. Modulating *TCF7L2* expression through therapy may be a potential avenue for the treatment of T2D with a *TCF7L2* risk variant. Further research is necessary to determine the amount of *TCF7L2* variant participation in complicated regulatory functions. The relevance of ethnic differences has not yet been defined, even though the genetic contribution of *TCF7L2* to T2D has been confirmed in numerous populations[11].

In T2D group, our correlation and regression analysis revealed a substantial positive association between the expression of the *ADIPOQ* gene and expression of the *TCF7L2* gene. Additionally, recent data suggest that the Wnt pathway-related metabolic consequences connected to insulin resistance and T2D are complexly regulated by the *TCF7L2* gene and its expression in adipose tissue. The regulatory function of *TCF7L2* expression can vary for being in preadipocytes or mature adipocytes, according to a recent research by Chen *et al.*[29]. Increased beta-catenin binding in preadipocytes caused by *TCF7L2* overexpression promotes Wnt activation and prevents adipogenesis. On the other hand, downregulation of the *TCF7L2* gene stimulates the Wnt pathway in mature adipocytes with low beta-catenin levels, causing adipocyte hypertrophy, increased fat deposition in the liver, and insulin resistance[29]. In human adipose tissue, the expression levels of *TCF7L2* and adiponectin were positively correlated. In adipose tissues, *TCF7L2* might be a cause of increased adiponectin expression. This shows that *TCF7L2* may regulate the expression of adiponectin and that

TCF7L2 polymorphisms linked to an increased risk of T2D may have an impact on adiponectin levels *via* this regulatory mechanism. However, more studies are required to completely apprehend the association of *TCF7L2* with adiponectin expression.

Conclusion

This study concludes that there is increased expression of the *ADIPOQ* and *TCF7L2* genes in T2D subjects as compared to control group. In T2D group, a significant positive relationship was found between expression of the *ADIPOQ* gene and SBP, RBG and expression of *TCF7L2* gene. Also, a significant negative correlation was found between expression of the *TCF7L2* gene and DBP. The *ADIPOQ* gene expression and *TCF7L2* gene expression are independent predictors of T2D indicating their role in the development of T2D. Additional research is required to completely understand the mechanisms involved in the increased gene expression of *ADIPOQ* and *TCF7L2* in T2D.

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References

1. Kolarić V, Svirčević V, Bijuk R, Zupančič V. Chronic complications of diabetes and quality of life. Acta Clin Croat. 2022;61(3):520-7. doi: 10.20471/acc.2022.61.03.18.
2. Cui M, Gao Y, Zhao Y, Pang H, Chen L, Wang Z, Zhao L, Li M. Association between adiponectin gene polymorphism and environmental risk factors of type 2 diabetes mellitus among the Chinese population in Hohhot. Biomed Res Int. 2020;2020:6383906. doi: 10.1155/2020/6383906.
3. Klisic A, Radoman Vujacic I, Munjas J, Ninic A, Kotur-Stevuljevic J. Micro-ribonucleic acid modulation with oxidative stress and inflammation in patients with type 2 diabetes mellitus – a review article. Arch Med Sci. 2022;18(4):870-80. doi: 10.5114/aoms/146796.
4. Begum M, Choubey M, Tirumalasetty MB, Arbee S, Mohib MM, Wahiduzzaman M, Mamun MA, Uddin MB, Mohiuddin MS. Adiponectin: a promising target for the treatment of diabetes and its complications. Life (Basel). 2023;13(11):2213. doi: 10.3390/life13112213.
5. Duval A, Busson-Leconiat M, Berger R, Hamelin R. Assignment of the TCF-4 gene (TCF7L2) to human chromosome band 10q25.3. Cytogenet Cell Genet. 2000;88(3-4):264-5. doi: 10.1159/000015534.
6. Duval A, Rolland S, Tubacher E, Bui H, Thomas G, Hamelin R. The human T-cell transcription factor-4 gene: structure, extensive characterization of alternative splicings, and mutational analysis in colorectal cancer cell lines. Cancer Res. 2000;60(14):3872-9.
7. Grant SF, Thorleifsson G, Reynisdottir I, Benediktsson R, Manolescu A, Sainz J, *et al*. Variant of transcription factor 7-like 2 (TCF7L2) gene confers risk of type 2 diabetes. Nat Genet. 2006;38(3):320-3. doi: 10.1038/ng1732.
8. Sladek R, Rocheleau G, Rung J, Dina C, Shen L, Serre D, *et al*. A genome-wide association study identifies novel risk loci for type 2 diabetes. Nature. 2007;445(7130):881-5. doi: 10.1038/nature05616.
9. Wang J, Kuusisto J, Vänttinen M, Kuulasmaa T, Lindström J, Tuomilehto J, *et al*. Variants of transcription factor 7-like 2 (TCF7L2) gene predict conversion to type 2 diabetes in the Finnish Diabetes Prevention Study and are associated with impaired glucose regulation and impaired insulin secretion. Diabetologia. 2007;50(6):1192-200. doi: 10.1007/s00125-007-0656-6.
10. da Silva Xavier G, Loder MK, McDonald A, Tarasov AI, Carzaniga R, Kronenberger K, *et al*. TCF7L2 regulates late events in insulin secretion from pancreatic islet beta-cells. Diabetes. 2009;58(4):894-905. doi: 10.2337/db08-1187.
11. Norton L, Chen X, Fourcaudot M, Acharya NK, DeFronzo RA, Heikkinen S. The mechanisms of genome-wide target gene regulation by TCF7L2 in liver cells. Nucleic Acids Res. 2014;42(22):13646-61. doi: 10.1093/nar/gku1225.
12. Geoghegan G, Simcox J, Seldin MM, Parnell TJ, Stubben C, Just S, *et al*. Targeted deletion of Tcf7l2 in adipocytes promotes adipocyte hypertrophy and impaired glucose metabolism. Mol Metab. 2019;24:44-63. doi: 10.1016/j.molmet.2019.03.003.
13. Tsao DA, Huang CW, Chang HR, Hsieh KC, Tung TC, Liao MC, *et al*. Single nucleotide polymorphism of TCF7L2 and adiponectin genes for type 2 diabetes mellitus in Taiwan. Fooyin J Health Sci. 2009;1(1):41-7.
14. Banfi G, Salvagno GL, Lippi G. The role of ethylenediamine tetraacetic acid (EDTA) as *in vitro* anticoagulant for

diagnostic purposes. Clin Chem Lab Med. 2007;45(5):565-76. doi: 10.1515/CCLM.2007.110.

15. Fève B, Bastard JP. The role of interleukins in insulin resistance and type 2 diabetes mellitus. Nat Rev Endocrinol. 2009;5(6):305-11. doi: 10.1038/nrendo.2009.62.
16. Abbas E, Ahmed Siddiqui I, Khan MS, Perveen K, Butt A, Fawwad A. Fasting glucagon level in type 2 diabetes and impaired glucose tolerance and its association with diabetes-associated clinical parameters: a study from Karachi, Pakistan. Cureus. 2021;13(2):e13430. doi: 10.7759/cureus.13430.
17. Bowen ME, Xuan L, Lingvay I, Halm EA. Random blood glucose: a robust risk factor for type 2 diabetes. J Clin Endocrinol Metab. 2015;100(4):1503-10. doi: 10.1210/jc.2014-4116.
18. Kosekli MA, Aktas G. Serum uric acid to HDL cholesterol ratio is associated with diabetic control in new onset type 2 diabetic population. Acta Clin Croat. 2023;62(2):277-82. doi: 10.20471/acc.2023.62.02.04.
19. Klisic A, Kocic G, Kavaric N, Jovanovic M, Stanisic V, Ninic A. Body mass index is independently associated with xanthine oxidase activity in overweight/obese population. Eat Weight Disord. 2020;25(1):9-15. doi: 10.1007/s40519-018-0490-5.
20. Klisic A, Kocic G, Kavaric N, Jovanovic M, Stanisic V, Ninic A. Xanthine oxidase and uric acid as independent predictors of albuminuria in patients with diabetes mellitus type 2. Clin Exp Med. 2018;18(2):283-90. doi: 10.1007/s10238-017-0483-0.
21. Zhu W, Cheng KK, Vanhoutte PM, Lam KS, Xu A. Vascular effects of adiponectin: molecular mechanisms and potential therapeutic intervention. Clin Sci (Lond). 2008;114(5):361-74. doi: 10.1042/CS20070347.
22. Lihn AS, Pedersen SB, Richelsen B. Adiponectin: action, regulation and association to insulin sensitivity. Obes Rev. 2005;6(1):13-21. doi: 10.1111/j.1467-789X.2005.00159.x.
23. Xu A, Vanhoutte PM. Adiponectin and adipocyte fatty acid binding protein in the pathogenesis of cardiovascular disease. Am J Physiol Heart Circ Physiol. 2012;302(6):H1231-40. doi: 10.1152/ajpheart.00765.2011.
24. Saha A, Adak S, Chowdhury S, Bhattacharyya M. Enhanced oxygen releasing capacity and oxidative stress in diabetes mellitus and diabetes mellitus-associated cardiovascular disease: a comparative study. Clin Chim Acta. 2005;361(1-2):141-9. doi: 10.1016/j.cccn.2005.05.018.
25. Tang YH, Wang YH, Chen CC, Chan CJ, Tsai FJ, Chen SY. Genetic and functional effects of adiponectin in type 2 diabetes mellitus development. Int J Mol Sci. 2022;23(21):13544. doi: 10.3390/ijms232113544.
26. Alimi M, Goodarzi MT, Nekoei M. Adiponectin gene polymorphisms and risk of type 2 diabetes: an updated evidence for meta-analysis. Diabetol Metab Syndr. 2021;13(1):133. doi: 10.1186/s13098-021-00749-x.
27. Lyssenko V, Lupi R, Marchetti P, Del Guerra S, Orho-Melander M, Almgren P, *et al*. Mechanisms by which common variants in the TCF7L2 gene increase risk of type 2 diabetes. J Clin Invest. 2007;117(8):2155-63. doi: 10.1172/JCI30706.
28. Yi F, Brubaker PL, Jin T. TCF-4 mediates cell type-specific regulation of proglucagon gene expression by beta-catenin and glycogen synthase kinase-3beta. J Biol Chem. 2005;280(2):1457-64. doi: 10.1074/jbc.M411487200.
29. Chen X, Ayala I, Shannon C, Fourcaudot M, Acharya NK, Jenkinson CP, *et al*. The diabetes gene and wnt pathway effector TCF7L2 regulates adipocyte development and function. Diabetes. 2018;67(4):554-68. doi: 10.2337/db17-0318.

Sažetak

PROFIL EKSPRESIJE GENA *ADIPOQ* I *TCF7L2* KOD OSOBA S DIJABETESOM TIP 2 U PAKISTANU

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Cilj ove studije bio je ispitati ekspresiju gena *ADIPOQ* i transkripcijskog faktora 7-sličnog 2 (*TCF7L2*) kod stanovnika Pakistana oboljelih od dijabetesa melitusa tip 2 te pronaći potencijalnu vezu između ekspresije gena *ADIPOQ* i *TCF7L2* kod ovih ispitanika. Ova presječna studija obuhvatila je 200 osoba s dijabetesom tip 2 i 200 kontrolnih ispitanika. Analiza ekspresije gena *ADIPOQ* i *TCF7L2* provedena je pomoću polimerazne lančane reakcije u stvarnom vremenu. Značajno povećanje ekspresije gena *ADIPOQ* i *TCF7L2* zabilježeno je u skupini s dijabetesom tipa 2 u usporedbi s kontrolnom skupinom. U skupini s dijabetesom tip 2 utvrđena je značajna pozitivna korelacija između ekspresije gena *ADIPOQ* i sistoličkog krvnog tlaka, razine glukoze u krvi i ekspresije gena *TCF7L2*. Značajna negativna korelacija zabilježena je između ekspresije gena *TCF7L2* i dijastoličkog krvnog tlaka. Multivarijabilna linearna regresijska analiza pokazala je da su ekspresija gena TCF7L2 (b=0,274; prilagođena promjena R^2, 0,135; p=0,005) i ekspresija gena *ADIPOQ* (b=0,270; prilagođena promjena R^2, 0,148; p=0,001) pozitivno povezane kod dijabetesa tipa 2, dok je dijastolički krvni tlak (b=-0,296; prilagođena promjena R^2, 0,148; p=0,001) negativno povezan kod dijabetesa tip 2 u usporedbi s kontrolnom skupinom. Kada je provedena stepenasta linearna regresija uzimajući u obzir ekspresiju gena *TCF7L2* kao zavisnu varijablu u skupini s dijabetesom tip 2 izračunat je jedan model koji pokazuje ekspresiju gena *ADIPOQ* (b=0,335; p=0,001) kao važan prediktor dijabetesa tipa 2. Kada je korištena stepenasta linearna regresija uzimajući u obzir ekspresiju gena *ADIPOQ* kao zavisnu varijablu izračunata su dva modela koja pokazuju ekspresiju gena *TCF7L2* (b=0,335; p=0,001) i sistolički krvni tlak (b=0,138; p=0,041) kao važne determinante dijabetesa tipa 2. Dakle, ekspresija gena *ADIPOQ* i *TCF7L2* su nezavisni prediktori dijabetesa tipa 2. U zaključku, utvrđena je povećana ekspresija gena *ADIPOQ* i gena *TCF7L2* kod ispitanika s dijabetesom tipa 2 u usporedbi s kontrolnom skupinom. Ekspresija gena *ADIPOQ* i ekspresija gena *TCF7L2* predstavljaju nezavisne prediktore dijabetesa tipa 2, što ukazuje na njihovu ulogu u razvoju ove bolesti. Potrebna su buduća istraživanja kako bi se razjasnili mehanizmi uključeni u povećanu ekspresiju gena *ADIPOQ* i *TCF7L2* kod osoba s dijabetesom tip 2.

Ključne riječi: *Dijabetes tip 2; Gen ADIPOQ; Gen TCF7L2; Ekspresija gena; Pakistanska populacija*