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Research Article

Sanger Sequencing Technique Is Employed to Identify the Polymorphism of The Transcription Factor 7/Like2 (TCF7/L2) Gene Associated with Obesity, Dyslipidemia, Hypertension, And Diabetic Retinopathy in The Type 2 Diabetes Population Presenting to The Tertiary Care Unit in Karachi.

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Abstract

Introduction: Diabetes mellitus is a metabolic disorder characterized by hyperglycemia caused due to either decrease in insulin production or reduced effect of insulin on cells. Diabetic retinopathy (DR) is a chronic complication of diabetes mellitus which can be due to micro vascular changes occurring in retina due to hyperglycemia. In many studies, polymorphism in gene TCF7L2 rs7903146 has been shown to increase susceptibility to developing diabetes mellitus. Association between Diabetic retinopathy (DR) and polymorphism of TCF7L2 expression and alteration in VEGF expression level has also been found in certain population.

Aims and Objective: To find out the relationship between the polymorphism of the transcription factor 7/like2 (TCF7/L2) gene and diabetes retinopathy, hypertension, obesity, and dyslipidemia in the population of type 2 diabetics presenting to the Karachi tertiary care unit.

Material and Method: The study subjects were divided into three groups i.e. control group (74) Type 2 Diabetes mellitus (T2DM) patients with retinopathy (45) and T2DM patients without retinopathy (29). Early treatment diabetic retinopathy system (ETDRS) was used for the diagnosis of retinopathy.

Results: A total of 148 subjects were recruited, 74 in Group A, 25 in Group B and 49 in Group C. Mean age (years) of Group A, group B, and Group C was 43.27±9.45, 54.0±13.2 and 51.2±10.8, respectively. In all the groups, the male and female ratio were almost equal. Results for systolic blood pressure, fasting blood glucose, HbA1c, triglycerides,, and low-density lipoprotein cholesterol were found significantly high ($p<0.05$) between the groups. The TT genotype of TCF7/L2 rs 7903146 was observed to be more abundant in group B (0.80) than in group C (0.39) and group A (0.1). While the CT genotype was found higher in group A (0.77) and group C (0.59) than in group A (0.20). Moreover, the proportion of mutant T allele was predominantly higher in group B (0.90) and group C (0.68) than in group A (0.40) in retinopathy. The

majority of the T2DM patients with retinopathy had a significant presence of complications like retinal hemorrhages, microdot, and blindness.

Conclusion: Overall, the homozygous TT genotype of *TCF7L2 rs7903146* polymorphism was found dominant in T2DM subjects with retinopathy as compared with T2DM without retinopathy, and CT genotype was found higher in T2DM subjects without retinopathy and healthy individuals. We also found mutant T allele was predominantly higher in T2DM subjects with and without retinopathy.

Keywords; TCF7L2, T2DM, DR, PDR, Polymorphism TCF7L2.

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INTRODUCTION

Diabetes mellitus is a metabolic disorder characterized by chronic hyperglycemia due to either reduced insulin production or insufficient insulin action. Insulin insufficiency or absence causes metabolic anomalies in carbohydrate, lipids, and protein metabolism. According to the American Diabetes Association (ADA), diabetes mellitus is classified as Type 1, Type 2, and gestational diabetes mellitus (GDM)(1).Damage to the big vessels of the body like carotid arteries, coronary arteries, etc. causes macro vascular problems, which is known as Macroangiopathy. Atherosclerosis is the pathogenic process underlying macro vascular disease. (2). Vasoconstrictor hormone angiotensin II stimulates the oxidation of lipids from low-density lipoprotein (LDL) particles to oxidized LDL, which builds up at the arterial endothelial walls. This draws in immune cells like monocytes, which sets off an immunological reaction that damages big vessels both morphologically and functionally, leading to cardiovascular diseases like myocardial infarction. (3).Diabetic antipathy, or Small blood vessels damaged by diabetes mellitus like arterioles and capillaries is one of the chronic complication of type 2 diabetes mellitus. Environmental factors like oxidative stress, Polyol accumulation, and advanced glycation of end products (AGEs) contribute to development of diabetic angiopathy (4). Changes in vessel lining alter the endothelial permeability and blood flow in microvasculature, resulting in diabetic nephropathy, neuropathy, and retinopathy (5).

Macular degeneration or increased microvascular permeability are two causes of DM- induced retinal injury (6). Hyperglycemia, hypertension, ageing, and dyslipidemia are all potential risk factors for DR. Findings of retinal hemorrhages, cotton wool patches, proliferative retinal vessels and micro aneurysm are used as criteria in retinal examination for diagnose DR (7).

Diabetic ocular disease progresses to a more severe stage called PDR. This occurs in the retina and is called neovascularization. Patients who have a few bleeds may develop black patches or floaters. Yet, vision impairment and eventual blindness would result from numerous fragile blood vessels bleeding into the vitreous. Scar tissue may occur as a result of these new blood vessels. Macular deformities or a detached retina may result from scar tissue. Peripheral and central vision loss can result from PDR, which is a highly dangerous consequence**.**(8). the pathogenesis of DR include vessel occlusions, formation of micro aneurysms and angiogenesis. Vessel occlusions occur due to formation of hard exudates which are yellow- white extracellular lipid leakage from aberrant retinal capillaries leads to variable-sized intra retinal deposits (9). Micro aneurysms are deep red dots which are sometimes observed in the eye's posterior poles as a result of pericyte degeneration (10). Prolonged vessel occlusion can lead to retinal ischemia. Angiogenesis is induced by the obstruction of retinal arteries, resulting in diabetic macular edema (11). Visual impairment may result from fluid and protein accumulation in the macular area. (12).The Wnt are a family of secreted glycoprotein. There are two categories for the Wnt signaling pathway;1)Non-canonical pathway.2) Canonical pathway.

Transcription factor 7-like2 (TCF7L2) and Wingless/Integrated (Wnt) Signaling pathway and beta catenin

Wnt glycol proteins, bind with seven-Tran membrane domain frizzled receptors and the LRP5/6(low-density lipoprotein receptor-related proteins co receptors. After binding with receptor, Wnt signals are transmitted by an association between the Wnt receptors and Disheveled (Dvl), an event that triggers the disruption of the complex that contains adenomatous polyposis coli, axin, GSK-3, and β-cat, thus preventing the phosphorylation-dependent degradation of β-cat (13).After entering the nucleus, β-cat forms the β-cat/TCF complex, which

activates downstream target genes of β-cat/TCF (or Wnt). (14). These HMG box TCF proteins act as transcriptional repressors of the Wnt target genes in the nucleus in the absence of Wnt signaling. (15). When β-cat is not present, B-cat transforms TCF into a transcriptional activator for the same set of genes that TCF represses. The human TCF7L2 functional domains that interact with β-cat, DNA, and β-cat/TCF also interact with Smad4, a crucial mediator of signals generated by TGFβ growth factor super family members via TGFβ receptors. (16). Furthermore, via its interaction with TCF7L2, the HMG-box repressor HBP1 suppresses the Wnt signaling cascade. (17).

TCF7L2 Gene Polymorphism causes Diabetes, Dyslipidemia and hypertension.

When a population has many alleles at a given locus, this is known as polymorphism. This polymorphism can be preserved by striking a balance between mutation-induced variety and natural selection (18). There are three types of DNA polymorphisms: tandem repeat polymorphism, copy number variations, and signal nucleotide polymorphism (SNPs).

A DNA sequence variance that is caused by a single nucleotide is referred to as a single nucleotide polymorphism (SNP) such as adenine (A), thymine (T), cytosine (C), guanine (G). Gene Mutations such as insertions, deletions and recombination cause polymorphism((19)

The Wnt signaling pathway component transcription factor 7 like aids in the regulation ofcell proliferation and differentiation. The interaction of the Wnts receptor complex, which releases β-catenin, initiates Wnt signaling (20). B-Catenin enters nucleus binds with TCF7L2 gene (21). The TCF7L2 protein binds to the promoter region to help with gene expression after interacting with beta catenin in the nucleus. (22). Pro glucagon, a precursor of glucagon-derived hormones such as glucagon-like peptide-1, is expressed under the control of TCF7L2. (GLP-1). GLP-1 binding with Glucagon like peptide-1 receptor (GLP-1R).GLP-1R limits food intake by activating GLP-1R in the hypothalamus and stomach, It reduces plasma glucose levels and prevents the stomach intestine from emptying. Moreover, GLP-1R promotes the development of new cells, insulin production, and cardio protective effects. It also aids in the expression of glucagon – like peptide – 1 (GLP-1), vascular endothelial growth factor (VEGF), and intercellular adhesion molecule-1(ICAM-1).GLP-1 malfunction and insulin secretion are caused by the TCF7L2 polymorphism (23).Reduced GLP-1 effects result in decreased insulin secretion, insulin processing irregularities, decreased glucagon-like peptide-1 effects, impaired insulin secretion, and increased hepatic glucose production, all of which contribute to insulin resistance (24). TCF7L2 mRNA expression is significantly higher in the pancreatic islets of T allele carriers, and this has been associated with reduced production of insulin and incretion consequences. (25).

Association of TCF7L2 with Obesity and Dyslipidemia.TCF7L2 effect on T2D risk factor, which is modulated by obesity. Polymorphism of TCF7L2rs7903146, rs1225537&rs7901695 T allele associated with a nominal decrease in TCF7L2 expression in adipose tissue which causes increase obesity. Increased adiposity associated with high levels of plasma aldosterone. It is suggested that obesity activated by RAAS (renin-angiotensin-aldosterone system). Angiotensin II and aldosterone also promote insulin resistance through non-genomic mechanisms. Activation of serine kinases responsible for the increase level of serine phosphorylation, which may cause reduction in insulin receptor substrate protein 1, which causes impaired phosphatides inositol 3-kinase and protein kinase B stimulation (26).Impaired Phosphatidy inositol 3-kinase and protein kinase B stimulation diminished insulin metabolic signaling, and accumulation of nitric oxide in vessels which may causes in reduced endothelial-mediated vascular relaxation and the development of hypertension (27). TCF7L2 is expressed in adipose tissue which involves in Wnt/β-catenin signaling depend Ent regulation of adiposeness (28).LRP5/6isaFZD co receptor in Wnt/β catenin signaling, mutations of LRP6 showed increasing risk for coronary disease and metabolic disorders (29). Adiposetissue comprises various discrete depots, such as in guinal, inter scapular, peri gonadal retroperitoneal, and mesenteric depots, which are placed in defined position throughout the body (30).Visceral fat, insulin resistance, sedentary habits, and the environment are prevalent risk factors that are strongly associated with type 2 diabetes and hypertension**.** The polymorphism TCF7L2 rs7903146 increases susceptibility to diabetes due to impaired beta cell Defect & function are primarily associated with incident hypertension (31). Glycemic variability raises blood pressure via enhancing oxidative stress and causing chronic inflammation (32). One of the alternate explanations for the connection between hypertension and abnormalities in beta cell function is oxidative stress, which plays a significant role in glucose variations and blood pressure variability. (33).

Material and Method.

A Comparative study was designed at Department of Biochemistry, and Patients148 subjects were recruited from an affiliated institute of Baqai Medical university i.e. Baqai institute of diabeology & endocrinology (BIDE).Total 148 individuals of age group 18-50 years were recruited for this study. Questionnaire will be filled by the subject regarding their case history along with their dietary habits, physical activities, their weight, waist circumference. A written informed consent will be obtained from all patient enrolled in this study. Physical examination was carried out by taking patient history. Blood pressure, BMI and waist/hip ratio for each individual were measured .estimation of biochemical parameter fasting blood sugar and oral glucose tolerance test was done by GOD PAP, HbA1c was done by HPLC, lipid profile Cholesterol, Triglycerides, HDL, and LDL

was done enzymatic method. DNA was extracted by quaigen method. TCF7L2 gene 7901346 was detected by Sanger Sequencing Method. This method is based on the principle that single-stranded DNA molecules that differ in length by just a single nucleotide can be separated from one another using polyacrylamide gel electrophoresis, described earlier. One di deoxynucleotide, either ddG,ddA, ddc, or ddT.

Sanger sequencing is a six-step process.

1. Double-stranded DNA (dsDNA) is denatured and converted into single strands
2. Primer that matches one end of the sequence.
3. Four polymerase solutions containing four different ddNTP kinds but only one Anthropometric measurement ddNTP are type
4. Continues until a termination nucleotide is integrated at random.
5. The resultant DNA fragments denature to become single-stranded DNA.
6. The sequencing is ascertained when the denatured fragments are separated via gel electrophoresis.
7. Primer was design by NCBI

Participants were divided into three groups.

- Group A (Control group); Non diabetic without retinopathy;
- Group B Diabetic type 2 With retinopathy
- Group C Diabetic type 2 without retinopathy

Result

We observe that there was a significant ($p<0.05$) increase Fasting blood sugar (FBG) and Glycosylated haemoglobulin (HbA1c) between the groups. Fasting blood sugar (FBS) measurements for Group B patients with retinopathy (146.33 ±18.69), Group C patients without retinopathy (135.06±22.53), and Group C patients in control (91.54±11.86). Oral glucose tolerance test (OGTT) in control for two hours (119.51±23.4). HbA1c was determined to be 5.7±0.5 in the control group, 9.2±2.1 in the group B patient with retinopathy, and 8.6±2.7 in the group C patient without Serum levels of fasting serum sugar and Oral glucose tolerance test (OGTT) and glycosylated hemoglobin (HBA1c) levels were estimated in each individual of control, T2DM patients with retinopathy, and T2DM patients without retinopathy. Estimation of Lipid Profile (cholesterol, triglycerides, high-density lipoprotein (HDL),and low-density lipoprotein (LDL) were made in both control groups and T2DM patients with and without retinopathy**.** triglycerides and LDL cholesterol were found significant ($p<0.05$) between the groups. Measurement of total cholesterol (TC), Group A control (181.1±34.8,), Group B patient with retinopathy (178.2±47.9), Group C patient without retinopathy 189.6±44.3) and Triglycerides between the Group A control groups (181.4±120.1), Group B T2DM patients with (229.7± 35.1) and without retinopathy (208.1±36.56).In contrast. We found HDL in Group A control (26.6.±7.39), Group B patient with retinopathy (32.33±6.95), patient without retinopathy (34.93±8.54) and LDL was observed in control (104.27±31.6), Group B T2DM patients with (32.333±6.95) and Group c without retinopathy (34.93±8.54). Frequency distributions of baseline retinopathy characteristics in T2DM subjects with and without retinopathy are presented in table 4. Characteristics of register blindness (24%), microdot (32%), blot hemorrhage (40%), exude hard (48%), exude soft (44%), and vitreous hemorrhage (28%) were commonly observed in diabetic subjects with retinopathy.

Genetic analysis DNA extracted and amplified product wereselected for Sanger sequencing.

Interpretation of gene sequencin*g*

Genotyping of TCF7/L2 rs 7903146 variant of group A, B and C shown in figure 2(a), 2(b) and 2(c), respectively.TCF7/L2 genetic variant rs 7903146 between group A and B was explored in table II. The TT genotype of this variant was observed to be abundant in group B (0.80) than group A (0.1). While the CT variant was found higher in group A (0.77) in comparison to group B (0.20). Moreover, the proportion of mutant T allele was predominantly higher in group B (0.90) than group A (0.40). Chi-square revealed a strong significant association of this variant ($\chi2= 6.447$, $p=0.011$). A significant role of mutant T allele was also found by odds ratio ($OR=0.07$, $p<0.001$). TCF7/L2 genetic variant rs 7903146 between group A and C was presented in table III. In group A (0.77) and group C (0.59) the CT genotype of this variant was found higher. Moreover, in group C, genotype variant TT (0.39) was also significantly found higher than in group A (0.1). The proportion of mutant T allele was also predominantly found higher in group C (0.68) than group A (0.40). Table V shows the TCF7/L2 genetic variant rs 7903146 between group B and C. The TT variant in group B (0.80) was found higher than group C (0.39). In group C, the CT genotype as compared to CC genotype was found dominant. The proportion of mutant T allele was predominantly found higher in group B (0.90) than group C (0.68)

Table 1: Comparative studies of FBS, Oral glucose tolerance test and HbA1c levels in T2DM patients with and without retinopathy compared to the control group

	Control N=74	**Patient with retinopathy N=25**	**Patient without retinopathy N= 49**	**P= value**
FBS (mg/dl)	91.54±11.86	146.33 ±18.69*	135.06±22.53	0.001*
2-hour GTT (mg/dl)	119.51±23.4	----	------	---
HbA1c (%)	5.7±0.5	9.2±2.1*	8.6±2.7	0.000*

*** Correlation is significance at the <0.05***

*** ANNOVA applied for Parameters**

Table 2: Comparative studies Cholesterol, Triglycerides, HDL and LDL in T2DM patients with and without retinopathy compared to the control group.

	Control N=74	**Patient with retinopathy N=25**	**Patient without retinopathy N= 49**	**P= value**
Cholesterol	181.1±34.8	178.2±47.9	189.6±44.3	0.699
Triglycerides	181.4±20.1	229.7± 35.1*	208.1±36.56	0.017*
HDL	26.6±7.39	32.33±6.95	34.93±8.54	0.065
LDL	104.27±31.6	152.0±39.96*	141.95±36.61	0.000*

*** Correlation is significance at the <0.05***

*** ANNOVA applied for Parameters**

Table 3: Frequency of retinopathy in T2DMwith and without retinopathy

Variable	**Diabetics withRetinopathy**
Register Blindness	24%
Microdot	32%
Blot Hemorrhage	40%*
Exude Hard	48%*
Exude Soft	44%*
Vitreous Hemorrhages	28%

*** Correlation is significance at the <0.05***

*** ANNOVA applied for Parameters**

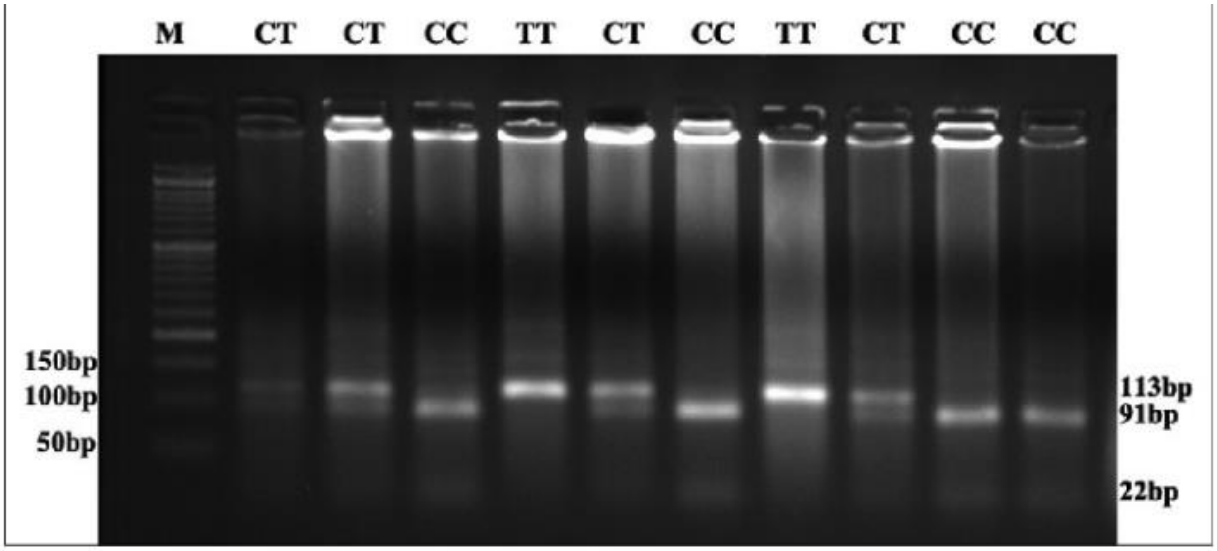
Table 4 (a) Comparative studies on Genotype of TCF7/L2 rs 7903146 variant distribution between Group A and group B

Genotype n = 99	**Group A n = 74**	**Group B n = 25**	**Chi-square p-value**
CC	16(0.22)*	0(0.0)	
CT	57(0.77)*	5(0.20)	6.447
TT	1(0.1)	20(0.80)*	p = 0.011
Alleles n = 198	**Group A n =148**	**Group B n = 50**	**OR (95% CI) p-value**
C	89(0.60)*	5(0.10)	0.07 (0.027, 0.1964) p<0.001
T	59(0.40)*	45(0.90)	

*** Correlation is significance at the <0.05***

*** ANNOVA applied for Parameters.**

P<0.05 denotes significance in data expressed as a number (percentage) and odd ratio (95% CI). To ascertain the relationship between the variables, the chi-square test and odds ratios with 95% confidence intervals (95% CI) were computed. Group A consists of healthy people, whereas Group B consists of T2DM patients with retinopathy.



Representative gel picture showing different genotypes of TCF7L2 gene polymorphism. Lane 1 is 50 bp DNA ladder; Lane 2, 3, 6, and 9 showing CT genotype; Lane 4, 7, 10, and 11 showing CC genotype; and Lane 5 and 8 showing TT genotype

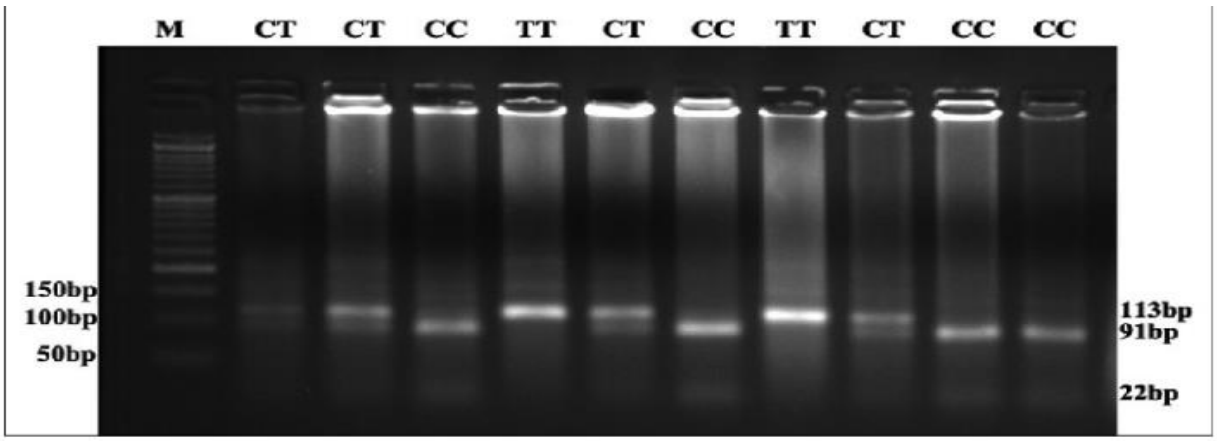
Table 4 (b) Comparative studies of Genotype of TCF7/L2 rs 7903146 variant distribution between group A and group C

Genotype n = 99	**Group A n = 74**	**Group C n = 49**	**Chi-square p-value**
CC	16(0.22)*	1(0.2)	69.751 p=0.002
CT	57(0.77)*	29(0.59)	
TT	1(0.1)	19(0.39)*	
Alleles n = 198	**Group A n =148**	**Group C n = 98**	**OR (95% CI) p-value**
C	89(0.60)*	31(0.32)	0.31(0.179 , 0.52) p<0.001
T	59(0.40)	67(0.68)*	

Correlation is significance at the <0.05

*** ANNOVA applied for Parameters**

Plotting the data as an odd ratio (95% CI) and number (proportion) shows significance at p<0.05. The relationship between the variables was ascertained using the chi-square test and odds ratios with 95% confidence intervals (95% CI). Group C consists of T2DM patients without retinopathy; Group A consists of healthy individuals.



Representative gel picture showing different genotypes of TCF7L2 gene polymorphism. Lane 1 is 50 bp DNA ladder; Lane 2, 3, 6, and 9 showing CT genotype; Lane 4, 7, 10, and 11 showing CC genotype; and Lane 5 and 8 showing TT genotype

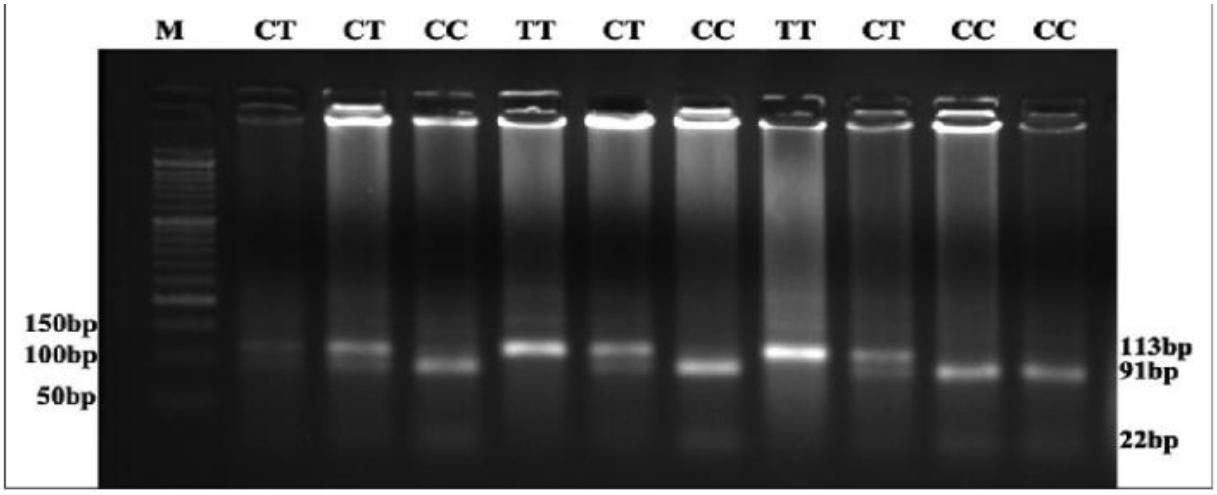
Table 4(c) Comparative Studies on Genotype of TCF7/L2 rs 7903146 variant distribution between group B and group C

Genotype n = 74	**Group B n = 25**	**Group C n = 49**	**Chi-square, p-value**
CC	0(0.0)	1(2.0)	11.38, p=0.003
CT	5(0.20)	29(0.59)*	
TT	20(0.80)*	19(0.39)	
Alleles n = 148	**Group B n = 50**	**Group C n = 98**	**OR (95% CI) p-value**
C	5(0.10)	31(0.32)*	0.2401(0.0868 , 0.6642) p<0.006
T	45(0.90)	67(0.68)*	

*** Correlation is significance at the <0.05***

*** ANNOVA applied for Parameters**

The data is shown as an odd ratio (95% CI) and a number (percentage); p<0.05 denotes significance. To find the relationship between the variables, the chi-square test and odds ratios with 95% confidence intervals (95% CI) were computed. Groups A and C: Group C consists of T2DM patients without retinopathy; Group A consists of healthy individuals.



Representative gel picture showing different genotypes of TCF7L2 gene polymorphism. Lane 1 is 50 bp DNA ladder; Lane 2, 3, 6, and 9 showing CT genotype; Lane 4, 7, 10, and 11 showing CC genotype; and Lane 5 and 8 showing TT genotype

Fig: Graphic representation shows genetic C/T Mutation in Control. (F)

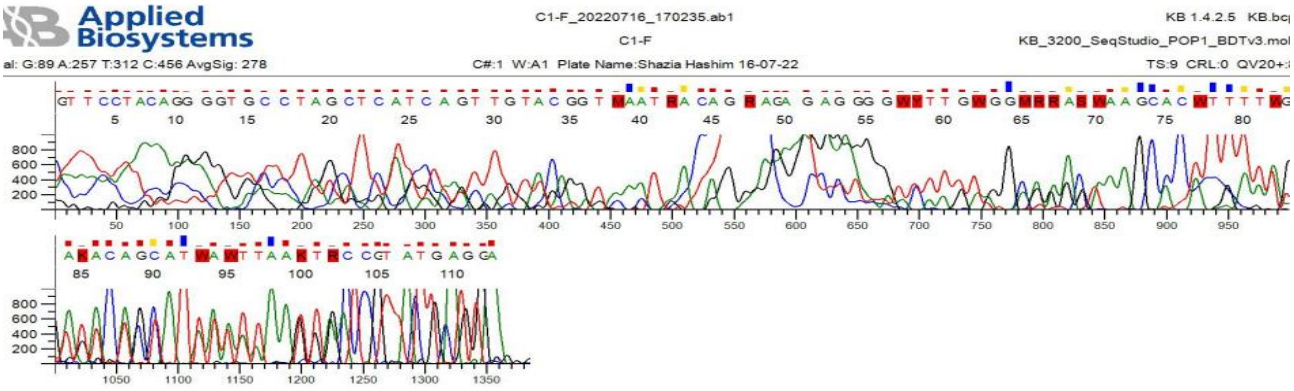
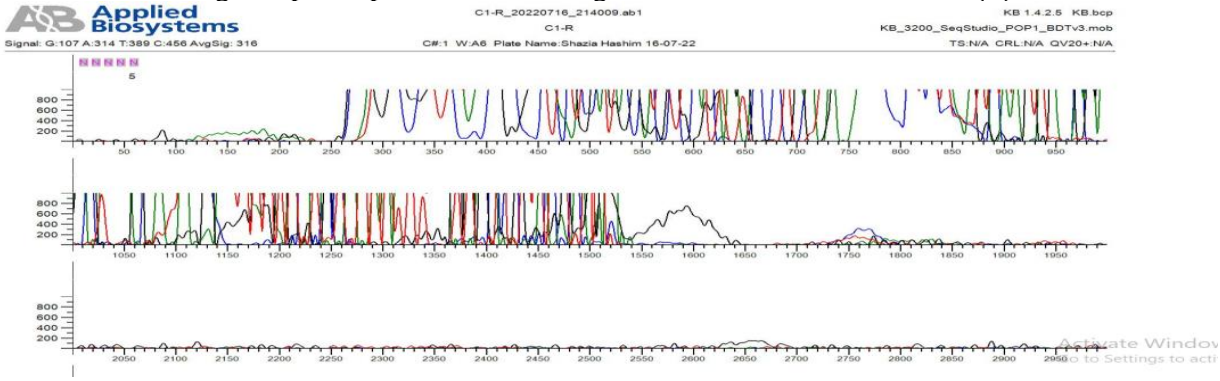
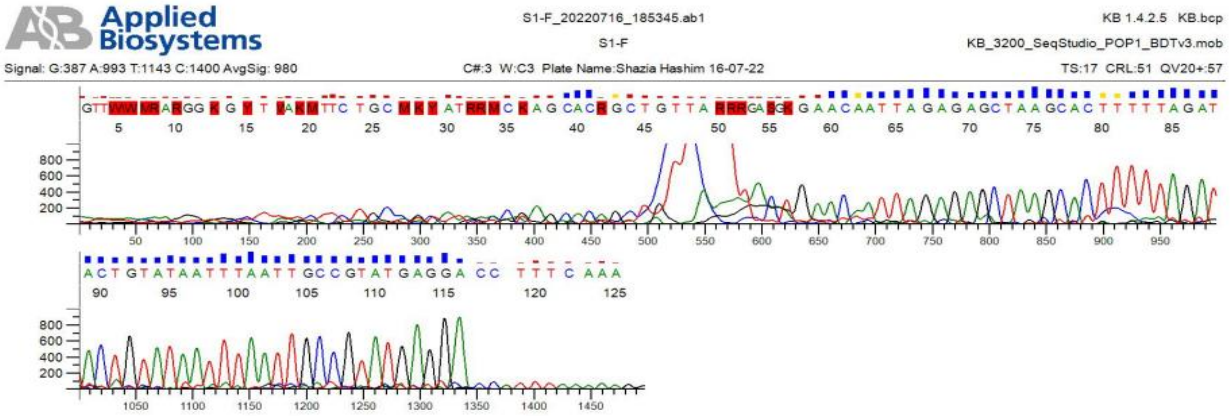


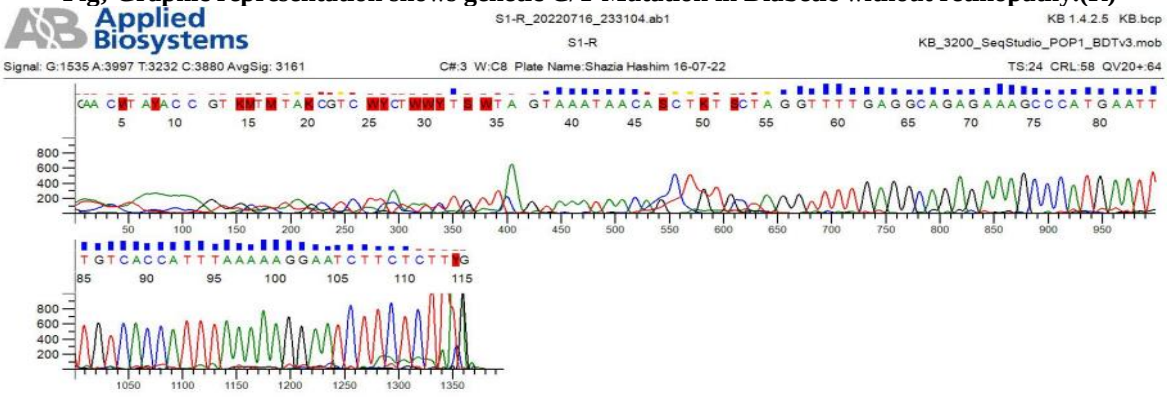
Fig: Graphic representation shows genetic C/T Mutation in Control. (R)



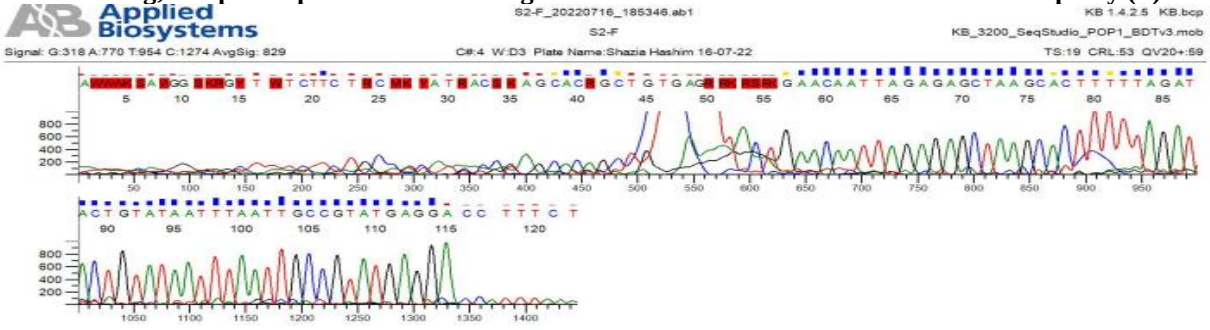
Fig; Graphic representation shows genetic C/T Mutation in Diabetic without retinopathy.(F)



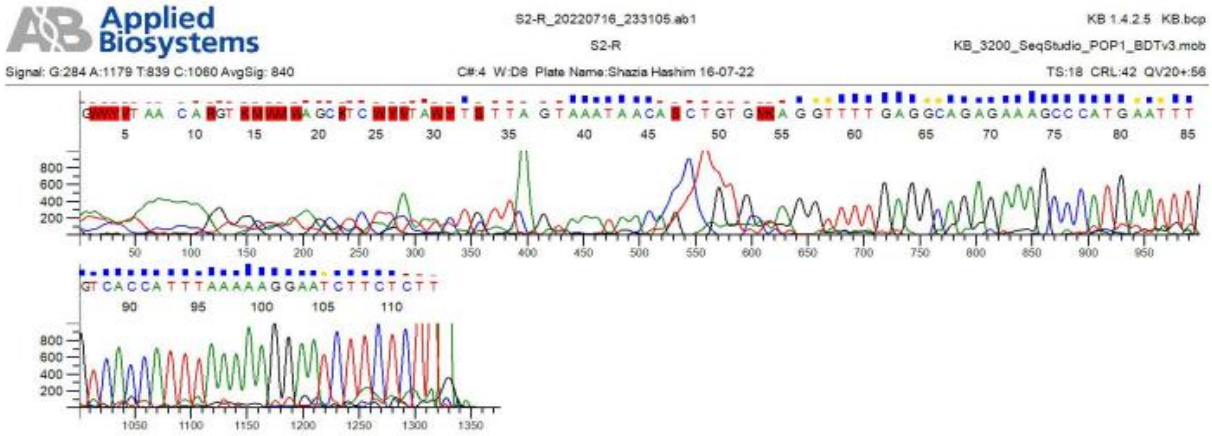
Fig; Graphic representation shows genetic C/T Mutation in Diabetic without retinopathy.(R)



Fig; Graphic representation shows genetic TT Mutation in Diabetic without retinopathy.(F)



Fig; Graphic representation shows genetic TT Mutation in Diabetic without retinopathy.(R)



DISCUSSION

Diabetes mellitus (T2DM) is a complicated metabolic disease linked to a number of environmental risk factors as well as genetic predisposition. The homozygous TT genotype of the TCF7L2 rs7903146 polymorphism was found to be dominant in T2DM people with retinopathy, while the CT genotype was found to be greater in both healthy individuals and T2DM subjects without retinopathy. The mutant T allele was also observed to be primarily higher in T2DM individuals, both with and without retinopathy. Significant differences were also seen between the groups for triglycerides, LDL, HbA1c, FBG, and SBP..

Our findings with dominant frequency of mutant T alleles in T2DM subjects with and without retinopathy are somehow in line with Beheld in et al study who also reported that TT genotype play role in development of T2DM(34). In literature, very scarce of data for TCF7L2 rs7903146 polymorphism in T2DM subjects with retinopathy was found, that make difficult to compare our data with other ethnic groups (35),(36). However, considering T2DM subjects without retinopathy our study also in line with Cropano et al who showed that the T risk allele confers the strongest risk of T2DM known to date in Caucasians and other ethnic groups (37) The occurrence of T alleles in subjects with T2DM is also consistent with Anjum et al and Assmann et al study who also found a significant association of CT and TT polymorphisms (38),(39). In meta-analysis of different ethnic groups a positive association of the CT genotype with T2DM was also reported (40).

We found poor control of glycaemia, triglycerides and LDL more prominent in T2DM subjects with retinopathy. Ahmed et al., reported that poor glycemic control is highly associated with DR (41). Our findings are in agreement with Gun avathy et al study who reported dyslipidemia in T2DM subjects (41). Our findings support Dornan et al., study who first time reported an association between DR and LDL cholesterol (42). It support the postulate that rise in viscosity and alterations in the fibrin lytic system which forms hard exudates occur with dyslipidemia. Additionally, alteration in membrane fluidity and leakage of plasma occur with incorporation of TG into the cell membrane that results in retinal hemorrhage and endothelial dysfunction which also worsens the retinopathy (43].

We observed most of the individuals were obese in all the groups though the findings are non-significant. It may because the prevalence of obesity was found with rising trends in Pakistan (44). In addition, it is well-established that a number of systemic disorders, including hypertension, stroke, dyslipidemia, and DR, are at risk due to obesity. (45).

We observed significantly high SBP in T2DM subjects with and without retinopathy. Recently, it was also reported that hypertension is an independent risk factor and strongly associated with DR (46).

High blood pressure and diabetes may cause optic neuropathy, systemic disorders, hemorrhages, blur vision, congestion retinal veins, and also form hard exudates that deposit in the macula that harm the retina including by reducing the inner retina and impairing the microcirculation (47.). Hypertension and diabetes are also responsible for focal arteriolar narrowing. The adipogenic development of pericytes, which are contractile cells found in small retinal arterioles, is inhibited by the Wnt/β-catenin/T-cell factor (TCF) (canonical) signaling pathway. This has subsequent effects on regulating retinal microvascular function (48). This pathway also controls the proliferation of smooth muscle cells in the blood vessels; it may also play a role in thickening, hyperplasia, and arteriosclerosis, which can lead to focal arteriolar constriction (49].

Small sample size as per our population due to low resources is the limitation of our study. In literature, TCF7L2 rs7903146 polymorphism with retinopathy in our population was not found is our study strength. Future research on large sample size to determine an association of TCF7L2 rs7903146 polymorphisms with DR and other associated risk factors are highly recommended for this population. In the present study T2DM patients with or without retinopathy were found to be significantly hypertensive when compared with

control group. These findings are in line with previous studies that show that hypertension is strongly associated with diabetic retinopathy (50). Hypertension and Diabetes mellitus may cause optic neuropathy showing signs of hemorrhages, blur vision, congestion of retinal veins, and also form hard exudates that deposit in the macula that harm the retina, including by impairing the microcirculation. (51).

Hypertension and diabetes mellitus are responsible for focal arteriolar narrowing. The canonical signaling pathway of Wnt/β-catenin/T-cell factor (TCF) may be involved in controlling the function of the retinal microvascular system. (52). this route controls the growth of vascular smooth muscle cells and may have a role in the development of arteriosclerosis and hyperplasia, which are characterized by focal arteriolar constriction. The blood vessel walls in the retina may thicken with elevated blood pressure. This could narrow blood vessels, which would prevent blood from getting to the retina. The retina may swell in certain circumstances. (53). The most common causes of hypertension retinal vein occlusion are blood clot development and artery hardening, or atherosclerosis. when elevated blood pressure harms the optic nerve by obstructing regular blood flow to the eyes. (54).

Molecular and biochemical mechanisms that have been implicated in diabetic retinopathyare increased flux of glucose through the Polyol and hexamine pathways, activation of protein kinase C, and increased formation of advanced glycation end product (55). When intracellular glucose levels rise, the Polyol pathway of glucose metabolism activates. Diabetes causes an increase in the activity of the sorbitol route in tissues that do not require insulin for cellular glucose uptake, such as the kidney, retina, peripheral nerves, and blood vessels. Because the sorbitol end product of the pylol route does not readily permeate across cell membranes, it builds up and damages the retina osmotically (56).

In the present study Fasting blood sugar, HBA1C and OGTT were significantly increased in T2DM patients with and without retinopathy compared to control group. This increase in glucose level for long time results in DR. In our study patients who develop retinopathy are those who are suffering from type 2 diabetes suffering since 11-16 years. Multiple studies have already established that the length of diabetes mellitus affects the incidence of diabetic retinopathy. Long-term diabetes mellitus lengthens the risk of developing retinopathy in those with type 2 diabetes. Patients with diabetes mellitus for 11–13 years get 41% retinopathy, compared to 60% in those with the disease for 14–16 years. It is already known that the buildup of glucose damages the blood vessels in the retina, which is the back of the eye, resulting in diabetic retinopathy. (57).Serum levels of triglycerides, HDLs, and LDLs are higher in T2DM individuals with diabetic retinopathy than in those without the condition. High levels of LDL and HDL were discovered to be linked with the severity and progression of diabetic retinopathy in earlier research. (58). Elevated levels of LDL and HDL lead to increased viscosity of blood and changes in the fibrinolytic system, resulting in the formation of hard exudates. Hyperlipidemia causes endothelial dysfunction due to reduced bioavailability of nitric oxide and breakdown of the blood retinal barrier, which leads to exudation of serum lipids and lipoproteins, resulting in DR changes (59). It is suggested in some studies that retinal hemorrhages caused by an elevated level of triglycerides (60). Our result is in accordance with these studies which shows significantly increase level of triglyceride in T2DM patients with diabetic retinopathy. A comparative study conducted in India, also revealed strong positive association between high triglyceride level and diabetic retinopathy. Genomic studies conducted in various ethnic populations reported that single-nucleotide polymorphism (SNP) in human TCF7L2 genes has a close relationship with occurrences of type 2 diabetes mellitus and its microvascular complications (61). In the present study, to find out an Association of Transcription factor 7/Like2 (TCF7/L2) Gene polymorphism with Obesity, Dyslipidemia, Hypertension and diabetic retinopathy in Type 2 Diabetic population reporting at Tertiary care unit of Karachi. We performed a case-control study to find out association between SNP polymorphisms with DR. Transcription factor 7-like 2 (TCF7L2) plays a crucial function in controlling basic processes like vascular development and pathological neovascularization in diabetes mellitus. TCF7L2 is also involved in the Wnt-signaling pathway, according to Ola, M. S., Nawaz, M., et al. (2013). Several studies have shown an extensive association between rs7903146 in TCF7L2, type 2 diabetes, obesity complications, dyslipidemia, and hypertension. (62).It is also proved in the previous study that in spite of high prevalence of the disease in South Asians, T2DM patients with the rs7903146 SNP of TCF7L2 are uncommon in Pakistani society (63).Another study which was conducted in Malaysia 2021 showed that C allele and CC genotype of *TCF7L2* (rs7903146) were significantly associated with increased risk of Diabetic retinopathy in T2DM patients investigated under recessive models. While dominant model showed no significant association with Diabetic retinopathy (64), In present study DNA extracted from blood sample of three different groups, and amplified by conventional PCR, the amplified DNAs were successfully sequenced by Sanger sequencing method. After PCR and subsequent sequencing all the obtained sequences were aligned with the reference sequence of TCF7L2 gene in the Gen bank to perform genotyping. Electropherogram were also analyzed carefully to spot any polymorphism (65). No polymorphism or C to T substitution was found in any of the study sample including cases and controls, all the samples were carrying CC genotype at rs7903146. Also, there was no allelic distribution for SNP rs7903146. The effect of polymorphism on different study variables could not be analyzed as no sample with rs7903146 polymorphism was detected (66) This type of result indicates that either

this type of polymorphism may not exist in our population or small sample size is the limitation of present study. We can extend our work to a bigger project with larger sample size to get a significant result.(67).

Limitation of study

1. **Insufficient Sample Size**: The study might have a small sample size, which can limit the power to detect significant genetic associations and increase the risk of Type I and Type II errors.
2. **Ethnic Diversity**: Genetic studies often face challenges related to population stratification, where differences in genetic backgrounds between subgroups can lead to confounding results. If the study population is ethnically homogeneous, the findings might not be applicable to more diverse populations

Future suggestion

- **Increase Sample Size and Diversity**
- **Follow-up Studies**: Design longitudinal studies to better understand causal relationships and temporal dynamics between genetic variants and phenotypes.
- **Whole-Genome Sequencing**: Utilize whole-genome sequencing to capture rare and novel genetic variants that may not be identified through genotyping arrays.
- **Epigenetics and Transcriptomics**: Explore epigenetic modifications and gene expression profiles to understand how genetic variants affect gene function and contribute to phenotypic outcomes.

CONCLUSION

Association to obesity, dyslipidemia, and stroke as well as the metabolic syndrome, Diabetes, hypertension, raised LDL and HDL levels causes of blood more viscous and alter the fibrin lytic system. This leads to the development of hard exudates that accumulate in the macula and damage the retina by decreasing the inner retina and affecting microcirculation. In type 2 diabetes individuals, the risk of developing retinopathy increases with prolonged diabetes mellitus.
It was also found that T2DM subjects with retinopathy along with obesity, dyslipidemia & hypertension had a homozygous TT genotype of the TCF7L2 rs7903146 polymorphism, while the CT genotype was stronger in healthy subjects and T2DM subjects without retinopathy. Furthermore, we found that the larger mutant T allele was preferentially carried by T2DM subjects with and without retinopathy.

REFRENCES

1. Accord Study Group and Accord Eye Study Group. (2010). Effects of medical therapies on retinopathy progression in type 2 diabetes. New England Journal of Medicine, 363(3), 233-244.
2. Acharya, S., Al-Elq, A., Al-Nafaie, A., Muzaheed, M., & Al-Ali, A. (2015). Type 2 diabetes mellitus susceptibility gene TCF7L2 is strongly associated with hyperglycemia in the Saudi Arabia Population of the eastern province of Saudi Arabia. Eur Rev Med Pharmacol Sci, 19(16), 3100-3106.
3. Al\Maskari, F., & El-Sadig, M. (2007). Prevalence of diabetic retinopathy in the United Arab Emirates: a cross-sectional survey. BMC ophthalmology, 7(1), 1-8.
4. Alnahdi, A., Alghamdi, A., Alkandari, K., Alsamir, A., & Alsomali, A. (2022),Retinopathies: Types, Etiologies, and Outcome.American Diabetes Association. (2005). Standards of medical care in diabetes. Diabetes care, 28(1), S4.
5. Aronow, W. S. (2001). Commentary on treatment of hypertension in the elderly,Cardiovascular drugs and therapy, 15(3), 281-282.
6. Begriche, K., Massart, J., Robin, M. A., Bonnet, F., & Fromenty, B. (2013). Mitochondrial adaptations and dysfunctions in nonalcoholic fatty liver disease. Hepatology, 58(4), 1497-1507.
7. Beloso, C., Souto, J., Fabregat, M., Romanelli, G., Javiel, G., & Mimbacas, A. (2018). Association of TCF7L2 mutation and atypical diabetes in a Uruguayan population. World Journal of Diabetes, 9(9), 157. 73
8. Bhavsar, A. R., Googe, J. M., Stockdale et al; Diabetic Retinopathy Clinical Research Network. (2009). Risk of endophthalmitis after intravitreal drug injection when topical antibiotics are not required: the diabetic retinopathy clinical research network laser-ranibizumab- triamcinolone clinical trials. Archives of ophthalmology, 127(12), 1581-1583.
9. Bos, M., & Agyemang, C. (2013). Prevalence and complications of diabetes mellitus in Northern Africa, a systematic review. BMC public health, 13(1), 1-7.
10. Bozkurt, L., Göbl, C. S., Tura, A., Chmelik, M., Prikoszovich, T., Kosi, L., & Kautzky- Willer, A. (2012). Fatty liver index predicts further metabolic deteriorations in women with previous gestational diabetes. PloS one, 7(2), e32710.
11. Cade, W. T. (2008). Diabetes-related microvascular and macrovascular diseases in the physical therapy setting. Physical therapy, 88(11), 1322-1335.
12. Callaghan, B. L., & Richardson, R. (2013). Early experiences and the development of emotional learning systems in rats. Biology of Mood & Anxiety Disorders, 3(1), 1- 7.
13. Chen, J., Stahl, A., Krah, N. M., Seaward, M. R., Dennison, R. J., Sapieha, P., & Smith,L. E. (2011). Wnt signaling mediates pathological vascular growth in proliferative retinopathy. Circulation, 124(17), 1871-1881.
14. Chen, X., Ayala, I., Shannon, C., Fourcaudot, M., Acharya, N. K., Jenkinson, C. P., & Norton, L. (2018). The diabetes gene and Wnt pathway effector TCF7L2 regulates adipocyte development and function. Diabetes, 67(4), 554-568. 74

15. Cheng, S. L., Ramachandran, B., Behrmann, A., Shao, J. S., Mead, M., Smith, C., & Towler, D. A. (2015). Vascular smooth muscle LRP6 limits arteriosclerotic calcification in diabetic LDLR−/− mice by restraining noncanonical Wnt signals. Circulation research, 117(2), 142-156.
16. Cherney, D. Z., Scholey, J. W., Sochett, E., Bradley, T. J., & Reich, H. N. (2011). The acute effect of clamped hyperglycemia on the urinary excretion of inflammatory cytokines/chemokines in uncomplicated type 1 diabetes: a pilot study. Diabetes care, 34(1), 177-180.
17. Chew, E. Y., Klein, M. L., Ferris, F. L., Remaley, N.et al , & Miller, D. (1996). Association of elevated serum lipid levels with retinal hard exudate in diabetic retinopathy: Early Treatment Diabetic Retinopathy Study (ETDRS) Report 22. Archives of ophthalmology, 114(9), 1079-1084.
18. Ciccacci, C., Di Fusco, D., Cacciotti, L., Morganti, R., D'Amato, C., Novelli, G., & Borgiani, P. (2013). TCF7L2 gene polymorphisms and type 2 diabetes: association with diabetic retinopathy and cardiovascular autonomic neuropathy. Acta diabetologica, 50(5), 789-799.
19. Clevers, H. (2006). Wnt/β-catenin signaling in development and disease. Cell, 127(3), 469-480.
20. Combs, T. P., & Marliss, E. B. (2014). Adiponectin signaling in the liver. Reviews in Endocrine and Metabolic Disorders, 15(2), 137-147.
21. Da Silva Xavier, G., Loder, M. K., McDonald, A., Tarasov, A. I., Carzaniga, R.,Kronenberger, K., & Rutter, G. A. (2009). TCF7L2 regulates late events in insulinsecreti.
22. Davoudi, S., & Sobrin, L. (2015). Novel genetic actors of diabetes-associated microvascular complications: retinopathy, kidney disease and neuropathy. The Review of Diabetic Studies: RDS, 12(3-4), 243.
23. IDF ATLAS 2021 (10th edition). Available https://diabetesatlas.org resource-files › 2021/07. [Last accessed on 11-4-23].
24. Viigimaa M, Sachinidis A, Toumpourleka M, Kouts ampasopoulos K, Alliksoo S, TitmaT. Macrovascular complications of type 2 diabetes mellitus Current vascular pharmacology. 2020;18(2):110-6.
25. Giri B, Dey S, Das T, Sarkar M, Banerjee J, Dash SK. Chronic hyperglycemia mediated physiological alteration and metabolic distortion leads to organ dysfunction, infection, cancer progression and other pathophysiological consequences: an update on glucose toxicity. Biomedicine & pharmacotherapy. 2018; 107:306-28.
26. Mrugacz M, Bryl A, Zorena K. Retinal vascular endothelial cell dysfunction and neuroretinal degeneration in diabetic patients. Journal of clinical medicine. 2021;10(3):458.
27. Kusuhara S, Fukushima Y, Ogura S, Inoue N, Uemura A. Pathophysiology of diabetic retinopathy: the old and the new. Diabetes & metabolism journal. 2018; 42(5):364-76.
28. Singh D, Tripathy K. Retinal Macroaneurysm. In Stat Pearls [Internet] 2021.
29. Liu J, Hu H, Qiu S, Wang D, Liu J, Du Z, et al. The Prevalence and Risk Factors of Diabetic Retinopathy: Screening and Prophylaxis Project in 6 Provinces of China. Diabetes, Metabolic Syndrome and Obesity: Targets and Therapy. 2022:2911-25. 76
30. Tokuda Y, Tabuchi H, Nagasawa T, Tanabe M, Deguchi H, Yoshizumi Y, et al. Automatic Diagnosis of Diabetic Retinopathy Stage Focusing Exclusively on Retinal Hemorrhage. Medicina. 2022;58(11):1681.
31. Lou L, Wang J, Wang J. Genetic associations between Transcription Factor 7 Like 2 rs7903146 polymorphism and type 2 diabetes mellitus: a meta-analysis of 115,809 subjects. Diabetology & Metabolic Syndrome. 2019;11(1):1-7.
32. Dong PP. Association of vascular endothelial growth factor expression and polymorphisms with the risk of gestational diabetes mellitus. Journal of clinical laboratory analysis. 2019;33(2):e22686.
33. Wu HH, Li YL, Liu NJ, Yang Z, Tao XM, Du YP, et al. TCF7L2 regulates pancreatic β-cell function through PI3K/AKT signal pathway. Diabe tology & Metabolic Syndrome. 2019;11(1):1-9.
34. Bahaaeldin AM, Seif AA, Hamed AI, Kabiel WA. Transcription factor 7-like-2 (TCF7L2) rs7903146 (C/T) polymorphism in patients with type 2 diabetes mellitus. Dubai Diabetes and Endocrinology Journal. 2020;26(3):112-8.
35. You H, Li Q, Kong D, Liu X, Kong F, Zheng K, et al. The interaction of canonical Wnt/β-catenin signaling with protein lysine acetylation. Cellular & Molecular Biology Letters. 2022;27(1):7.
36. Galderisi A, Tricò D, Pierpont B, Shabanova V, Samuels S, Dalla Man C, et al. A reduced incretin effect mediated by the rs7903146 variant in the TCF7L2 gene is an early marker of β-cell dysfunction in obese youth. Diabetes Care. 2020;43(10):2553-63.
37. American Diabetes Association. 2. Classification and diagnosis of diabetes: standards of medical care in diabetes—2019. Diabetes Care. 2019;42(1):13-28. 77
38. Azhar S, Khan FZ, Khan ST, Iftikhar B. Raised Glycated Hemoglobin (HbA1c) Level as a Risk Factor for Myocardial Infarction in Diabetic Patients: A Hospital-Based, Cross-Sectional Study in Peshawar. Cureus. 2022 Jun 7;14(6).
39. Basit A, Sabir S, Riaz M, Fawwad A. NDSP 05: Prevalence and pattern of dyslipidemia in urban and rural areas of Pakistan; a sub analysis from second National Diabetes Survey of Pakistan (NDSP) 2016–2017. J Diabetes Metab Disord. 2020;19(2):1215-25. doi.org/10.1007/s40200-020-00631-z
40. Pan WH, Yeh WT. How to define obesity?

Evidence-based multiple action points for public awareness, screening, and treatment: an extension of Asian-Pacific recommendations. Asia Pac J Clin Nutr. 2008;17(3):370–374.

41. Basit A, Tanveer S, Fawwad A, Naeem N, NDSP Members. Prevalence and contributing risk factors for hypertension in urban and rural areas of Pakistan; a study from second National Diabetes Survey of Pakistan (NDSP) 2016–2017. Clin Exp Hypertens. 2020;42(3):218-24.
42. Wangkumhang P, Chaichoompu K, Ngamphiw C, Ruangrit U, Chanprasert J, Assawamakin A, Tongsima S. WASP: a Web-based Allele-Specific PCR assay designing tool for detecting SNPs and mutations. BMC genomics. 2007 Dec;8:1-9.
43. Lobo I. Basic local alignment search tool (BLAST). Nature Education. 2008;1(1).
44. Bahaaeldin AM, Seif AA, Hamed AI, Kabiel WA. Transcription factor 7-like-2 (tcf7l2) RS7903146 (C/T) polymorphism in patients with type 2 diabetes mellitus. Dubai Diabetes and Endocrinology Journal. 2020 Dec 10;26(3):112-8.
45. Yan Y, Klein R, Heiss G, Girman CJ, Lange EM, Klein BE, Rose KM, Boerwinkle E, Pankow JS, Brancati FL, Ballantyne CM. The transcription factor 7-like 2 (TCF7L2) 78 polymorphism may be associated with focal arteriolar narrowing in Caucasians with hypertension or without diabetes: the ARIC Study. BMC endocrine disorders. 2010 Dec;10:1-0.
46. Cauchi S, Meyre D, Dina C, Choquet H, Samson C, Gallina S, Balkau B, Charpentier G, Pattou F, Stetsyuk V: Transcription factor TCF7L2 genetic study in the French population: expression in human beta-cells and adipose tissue and strong association with type 2 diabetes. Diabetes. 2006, 55: 2903-2908. 10.2337/db06-0474.
47. Cropano C, Santoro N, Groop L, Dalla Man C, Cobelli C, Galderisi A, et al The rs7903146 variant in the risk of prediabetes/type 2 diabetes in obese adolescents by impairing β-cell function and hepatic insulin sensitivity. Diabetes Care. 2017;40(8): ♣ 1082–9. https://doi.org/10.2337/dc17-0290.
48. Anjum N, Jehangir A, Liu Y. Two TCF7L2 variants associated with type 2 diabetes in the Han nationality residents of China. J Coll Physicians Surg Pak. 2018;28(10):794–797.
49. Assmann TS, Duarte GCK, Rheinheimer J, Cruz LA, Canani LH, Crispim D. The TCF7L2 rs7903146 (C/T) polymorphism is associated with risk to type 2 diabetes mellitus in Southern-Brazil. Arq Bras Endocrinol Metabol. 2014;58(9):918–25. https://doi.org/10.1590/0004-2730000003510.
50. Lou L, Wang J. Genetic associations between transcription factor 7 like 2 rs7903146 polymorphism and type 2 diabetes mellitus: a meta-analysis of 115,809 subjects. Diabetol Metab Syndr. 2019;11:56. https://doi.org/10.1186/s13098-019-0451-9. 79
51. Ahmed AM, Abbas I, Siddiqui SA. Hba1c Levels an Indicator of Glycemic Control in Diabetics With and Without Retinopathy. InProceedings 2023 Jan 23 (Vol. 37, No. 1, pp. 18-22).
52. Gunavathy N, Balaji R, Kumaravel V. Association of TCF7L2 Variants in Type 2 Diabetes Mellitus with Hypertriglyceridemia–A Case-Control Study. Indian Journal of Endocrinology and Metabolism. 2023 Jul 1;27(4):346-50.
53. Dornan TL, Carter RD, Bron AJ, Turner RC, Mann JI. Low density lipoprotein cholesterol: An association with retinopathy. Diabetologia. 1982;22:167–70. the severity of diabetic
54. Ezhilvendhan K, Sathiyamoorthy A, Prakash BJ, Bhava BS, Shenoy A.Association of dyslipidemia with diabetic retinopathy in Type 2 diabetes mellitus patients: A hospital-based study. Nov;13(Suppl 2):S1062. Journal of Pharmacy & Bioallied Sciences. 2021
55. Basit A, Askari S, Zafar J, Fawwad A, Members NDSP. NDSP 06: Prevalence and risk factors for obesity in urban and rural areas of Pakistan: A study from second National Diabetes Survey of Pakistan (NDSP), 2016–2017. Obes Res Clin Pract. 2021;15(1):19-25.
56. Piché ME, Tchernof A, Després JP. Obesity phenotypes, diabetes, and cardiovascular diseases. Circulation research. 2020;126(11):1477-500.
57. Bulum T, Tomić M, Vrabec R, Brkljačić N, Ljubić S. Systolic and diastolic blood pressure are independent risk factors for diabetic retinopathy in patients with type 2 diabetes. Biomedicines. 2023 Aug 10;11(8):2242. 80
58. Modi P, Arsiwalla T. Hypertensive retinopathy. InStatPearls [Internet] 2022. StatPearls Publishing.Nguyen H, Lee SJ, Li Y. Selective Activation of the Wnt Signaling Pathway as a Novel Therapy for the Treatment of Diabetic Retinopathy and Other Retinal Vascular Diseases. Pharmaceutics. 2022;14(11):2476.
59. Jridi I, Canté-Barrett K, Pike-Overzet K, Staal FJ. Inflammation and Wnt signaling: target for immunomodulatory therapy?. Frontiers in cell and developmental biology. 2021; 8:615131.
60. Dhaliwal, D. S., Radhakrishnan, S., Tsang, A., & Yang, Y. G. (2012). Nonfinancial disclosure and analyst forecast accuracy: International evidence on corporate social responsibility disclosure. The accounting review, 87(3), 723-759.
61. Ellis, D., Burgess, P. I., & Kayange, P. (2013). 'Teaching corner': Management of Diabetic Retinopathy. Malawi Medical Journal, 25(4), 116-120.
62. Ezhilvendhan, K., Sathiyamoorthy, A., Prakash, B. J., Bhava, B. S., & Shenoy, A. (2021). Association of dyslipidemia with diabetic retinopathy in type 2 diabetes mellitus patients: A hospital-based study. Journal of Pharmacy & Bioallied Sciences, 13 (Suppl 2), S1062.
63. Fatima, M. T., Bhat, A. A., Nisar, S., Fakhro, K.

A., & Akil, A. S. A. S. (2022). The role of dietary antioxidants in type 2 diabetes and neurodegenerative disorders: An assessment of the benefit profile. Heliyon, e12698.

64. Fishman, S. L., Sonmez, H., Basman, C., Singh, V., & Pore sky, L. (2018). The role of advanced glycation end-products in the development of coronary artery disease i patients with and without diabetes mellitus: a review. Molecular Medicine, 24(1), 1-12.
65. 65 Florez, J. C., Jablonski, K. A., Bayley, N., Pollin, T. I., de Bakker, P. I., Shuldiner,
a. A. R., & Altshuler, D. (2006). TCF7L2 polymorphisms and progression to diabetes
b. in the Diabetes Prevention Program. New England Journal of Medicine, 355(3), 241-250.
66. 66 Fowler, M. J. (2008). Microvascular and macro vascular complications of diabetes.
a. Clinical diabetes, 26(2), 77-82.

Aims and Objective of the study

- To find out an Association of Transcription factor 7/Like2 (TCF7/L2) Gene polymorphism with Obesity.
- To find out an Association of Transcription factor 7/Like2 (TCF7/L2) Gene polymorphism with Dyslipidemia, in Type 2 Diabetic population reporting at Tertiary care unit of Karachi.
- To find out an Association of Transcription factor 7/Like2 (TCF7/L2) Gene polymorphism with, Hypertension in Type 2 Diabetic population reporting at Tertiary care unit of Karachi.
- To find out an Association of Transcription factor 7/Like2 (TCF7/L2) Gene polymorphism with, diabetic with and without retinopathy in Type 2 Diabetic population reporting at Tertiary care unit of Karachi.