ORIGINAL RESEARCH ARTICLE

TCF7L2 GENE POLYMORPHISM AS A KEY RISK IN TYPE 2 DIABETES MELLITUSPATIENTS IN NORTH INDIAN POPULATION

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ABSTRACT

Transcription factor 7 like 2 gene (TCF7L2) has association with circulatory GLP-1 levels, metabolic disorders and type 2 diabetes mellitus (T2DM) in a couple of population. We aimed to determine the frequency distribution of rs7903146 gene polymorphism of TCF7L2, and its significance with the circulatory level of GLP-1 and other biochemical parameters in patients with T2DM and controls. This case-control study was conducted in 308 subjects (154 T2DM and 154 healthycontrols), aged between 25-75 years. Biochemical investigations performed included fasting and post prandial blood sugar level, lipid profile, LFT and KFT. Glycated haemoglobin (HbA1c) and circulatory GLP-1 levels were assayed using commercially available kits. PCR-RFLP method was used for genotyping. Mean levels of various biochemical parameters were significantly higherin T2DM than in healthy controls ($p<0.001$). The levels of circulatory GLP-1 was found significantly lower in T2DM as compared to healthy controls ($p<0.001$). There was significant association of CC and CT genotypes ($p= 0.016$) as well as CC and TT genotype ($p=0.042$) in T2DM patients. The frequency of the "T" allele of rs7903146 (C/T) polymorphisms was significantly higher in diabetic subjects (34.41%) compared to controls (24.35%). The present study confirmed a significant association of the TCF7L2 gene rs7903146 (C/T) polymorphism with a higher risk to T2DM in the North Indian population**.** It was observed that

TT genotype of rs7903146 has significant impact on circulatoryGLP-1 levels in T2DM cases.

KEYWORDS: TCF7L2, T2DM, SNP

INTRODUCTION

A major concern to human wellbeing is Type 2 diabetes mellitus (T2DM) owing to its widespread and continuously increasing incidence as well as the late circulatory complications it results in [1]. T2DM arises due to an interplay between genetic and epigenetic factors. It frequently has a significant genetic propensity, but this type of diabetes' genetic makeup is still unknown complicated and poorly understood [2]. According to Grant et al. in 2006, the gene encoding transcription factor 7-like 2 (TCF7L2) was substantially linked to T2DM [3]. The TCF7L2 gene, which encodes the fundamental transcription factor 4 (TCF-4), functions as a nuclear sensor in the Wnt signalling pathway [4]. The most significant component of the Wnt signalling system is β- catenin, which binds to TCF-4 to create the β-catenin/TCF-4 complex, which is a critical effector of the Wnt pathway and regulates the expression of hormone genes. This complex is essential for the genesis of pancreatic islets [5]. Given its associations with hyperglycemia, decreased insulin production, poor glucose tolerance, and physiological and architectural changes in human islets, the TCF7L2 rs7903146 risk allele has a significant impact on the beta cells in the islets of Langerhans [6].

Numerous studies have shown a substantial correlation between the risk of acquiring T2DM and the intronic single nucleotide polymorphism (SNP) rs7903146 (C/T) in the TCF7L2 gene [7]. However, data from North Indian population are scarce and inconsistent. Therefore, to forecast a representative future for the North Indian area as a whole, it is crucial to examine populations in the other Indian regions.

The aim of this study was to determine the frequency distribution of rs7903146 gene polymorphism of TCF7L2, and its association with the circulatory levels of GLP-1 and other biochemical parameters in patients with T2DM and controls.

MATERIALS AND METHODS

Subject Selection

All participants in this case-control study were recruited from the outpatient diabetes clinics of IIMS&R, Integral University, Lucknow (India), and K.G.M.U, King George's Medical University, Lucknow, along with healthy controls (India). Ethical clearance was obtained from Institution's ethical committee. A thorough clinical history was collected, which included the patient's age, sex, occupation, and socioeconomic position. Each individual gave written informed consent after being fully briefed about the study.

308 participants (154 T2DM and 154 healthy controls), ranging in age from 25 to 75, participated in the study. T2DM was classified using the World Health Organization's (2019) criteria, which include fasting blood sugar levels below 126 mg/dl and blood glucose loads below 200 mg/dl at two hours post-prandial. The study did not include subjects who were pregnant or using an oral

contraceptive, or those who had concurrent conditions including hypothyroidism, chronic liver disease, or those taking diuretics as well as those with these conditions. Additionally excluded from the study were participants with ischemic disease, angina, myocardial infarction (MI), and abnormal electrocardiograms.

Laboratory investigations

After an eight-hour fast, blood samples were taken from patients and controls in the morning. The antecubital vein was used to withdraw 5 mL of venous blood. Out of which 3ml was taken in EDTA/fluoride/plain vial for biochemical analysis and ELISA and 2ml was taken in EDTA vial for genomic study. Blood samples were centrifuged for ten minutes at 3000 rpm to separate the plasma. Laboratory investigations included liver function test (LFT), kidney function test (KFT), Lipid profile, Fasting blood sugar, Post parandial sugar and glycated haemoglobin (HbA1c) which were performed by fully automated autoanalyzer.

Estimation of serum GLP-1

GLP-1 serum levels were measured using an ELISA kit that is commercially available in the market (Krishgen Biosystems, India). The test was carried out twice in accordance with the manufacturer's instructions. GPL-1 had a CV of <12% between assays and a CV of <10% within assays. Its sensitivity; human GLP-1 frequently has a minimal detectable dosage of 5.22 pg/ml. The standard calibration range was 12.35 pg/ml - 1000 pg/ml

DNA extraction and Genotyping

The genomic DNA extraction kit from Qiagen was used to extract DNA from whole-blood samples. Using a Nanodrop 2000 (Thermoscientific) spectrophotometer, isolated genomic DNA was measured. Agarose gel electrophoresis was carried out under the Gel Dock system (Bio-Rad, Gel Doc XR+, Universal Hood II) to verify the purity of extracted DNA containing 10 mg/ml ethidium bromide. The TCF7L2 gene was genotyped using the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method, and the thermocycler (Applied Biosystems, UK) was used for amplification. The rs7903146 polymorphism was genotyped using the following primers:

forward,5′- CTGTTTCTTGCTTAGTCACTTTCTG -3′,

reverse 5′- CTTTCACTATGTATTGTTGCCAGTC -3′

The annealing temperature (Tm) was set using gradient PCR. At 58° C, the best band was attained. For the Polymerase Chain Reaction (PCR), a final reaction volume of 20µl was prepared, containing 100 ng of genomic DNA, 5 pmol of each primer, a premixed PCR master mix, reaction buffer made up of Tris Hcl -10 mM at pH 8.3, KCl -50 mM and MgCl2 -2mM as well as dNTPs were used at a concentration of 200 µM each, Taq polymerase at a concentration of 1.25 U, and 4µl of nuclease free water was added to make up the volume.

The following were the PCR reaction conditions: a 5 min initial denaturation at 95 °C, proceeded by 34 cycles of 30 s each of denaturation at 95 °C, annealing at 58 °C, for 30 s, extension at 72 °C, and a final extension step spanning 9 min at 72 °C. On a 2% agarose gel containing 10 µg/ml

ethidium bromide, PCR products were confirmed and viewed under a UV light. After PCR, the products were digested at 65°C for two hours using Hpy-CH4III restriction enzyme (Thermo scientific, USA). On a 2.5% agarose gel, the restriction fragments of the PCR products were split. Each run had a 50bp DNA ladder attached.

STATISTICAL ANALYSIS

Version 20.0 of the SPSS statistical package was used for all of the analysis (Armonk, NY, USA). ANOVA or an unpaired t-test were both used to compare all of the phenotypic data. Data were presented as mean SD (Standard Deviation). The t test was used to analyse the allelic and genotypic frequencies, which were presented with a 95% confidence interval (CI). Hardy-Weinberg equilibrium was evaluated in TCF7L2 rs7903146 genotypes. For all the data examined, a p value of less than 0.05 was regarded as statistically significant.

RESULTS

Anthropometric and biochemical characteristics of the study subjects

The anthropometric and biochemical parameters of the study subjects are displayed in Table 1. Between the case and control groups, age and gender were comparable (p= 0.27). Mean levels of anthropometric parameters i.e. systolic blood pressure (SBP), diastolic blood pressure (DBP) and body mass index (BMI) were significantly higher in T2DM cases as compared to controls with (p= < 0.0001), (p=0.0488) and (p=< 0.0001) respectively. Similarly, biochemical parameters such as blood sugar, HbA1c, total cholesterol, triglycerides, HDL, serum ALT, serum AST, potassium levels were also significantly raised in T2DM cases as compared to healthy controls (p<0.001). In comparison to healthy controls, there was a substantial increase in LDL and BUN levels in T2DM cases (p=0.0075 and p=0.0032, respectively). When compared to healthy controls, the levels of circulatory GLP-1 were observed to be considerably lower in people with T2DM (p<0.001).

Table 1: Anthropometric and Biochemical characteristics of Case (T2DM) and Control Groups

Parameters	**Case (n=154)**	**Control(n=154)**	**p-value**
AGE (years)	52.00 ± 9.97	53.32 ± 4.06	0.27
BMI ((kg/m^2)	28.52 ± 3.19	21.79 ± 1.89	< 0.0001*
SBP (mmHg)	132.05 ± 8.45	120.23 ± 8.91	< 0.0001*
DBP (mmHg)	80.03 ± 7.30	78.06 ± 6.23	0.0488*
FBS (mg/dl)	182.19 ± 31.08	80.02 ± 6.17	< 0.0001*
PPBS (mg/dl)	249.97 ± 35.21	112.54 ± 10.70	< 0.0001*
HbA1c (%)	7.65 ± 0.97	4.89 ± 1.23	< 0.0001*
Total Cholesterol (mg/dl)	135.07 ± 25.98	107.68 ± 11.27	< 0.0001*
Triglyceride (mg/dl)	121.56 ± 23.97	90.50 ± 11.69	< 0.0001*
HDL(mg/dl)	37.46 ± 9.01	46.76 ± 5.81	< 0.0001*
LDL (mg/dl)	81.11 ± 10.42	77.31 ± 8.69	0.0075*
Serum Creatinine (mg/dl)	0.83 ± 0.18	0.82 ± 0.20	0.73
BUN (mg/dl)	13.93 ± 3.07	12.70 ± 2.49	0.0032*

S. Bilirubin (mg/dl)	0.70 ± 0.255	0.66 ± 0.25	0.27
S.ALT (IU/L)	32.10 ± 10.22	23.16 ± 6.43	< 0.0001*
S.AST (IU/L)	30.83 ± 10.64	20.97 ± 6.12	< 0.0001*
S.ALP (IU/L)	71.05 ± 25.56	68.78 ± 16.90	0.48
K^+ (mEq/L)	4.14 ± 0.43	3.78 ± 0.16	< 0.0001*
Na^+ (mEq/L)	137.77 ± 2.35	137.52 ± 2.10	0.42
GLP-1 (pg/ml)	1.37 ± 0.98	2.81 ± 1.73	< 0.0001*

Values are presented as Mean Standard Deviation, with P< 0.05 deemed significant. FBS: Fasting Blood Sugar, PPBS: Post-Prandial Blood Sugar, HbA1c: Glycated Haemoglobin, SBP: Systolic Blood Pressure, DBP: Diastolic Blood Pressure, BMI: Body Mass Index, TC: Total Cholesterol, TG: Triglyceride, HDL: High-Density Lipoprotein, LDL: Low-Density Lipoprotein

Genotype and Allele distribution

The 700 bp PCR-amplified product was digested with the Hpy-CH4III restriction enzyme and then ran in 2.5% agarose gel electrophoresis to reveal the TCF7L2 polymorphism at rs7903146 (Fig. 1).

For the CC homozygote wild type, a fragment of 700 bp was found (absence of Hpy-CH4III restriction site). The three 700, 518, and 182 bp segments were present in the CT heterozygous. For the TT mutant homozygote, fragments of 518 bp and 182 bp were found. Analysis was done using a 50bp DNA ladder.

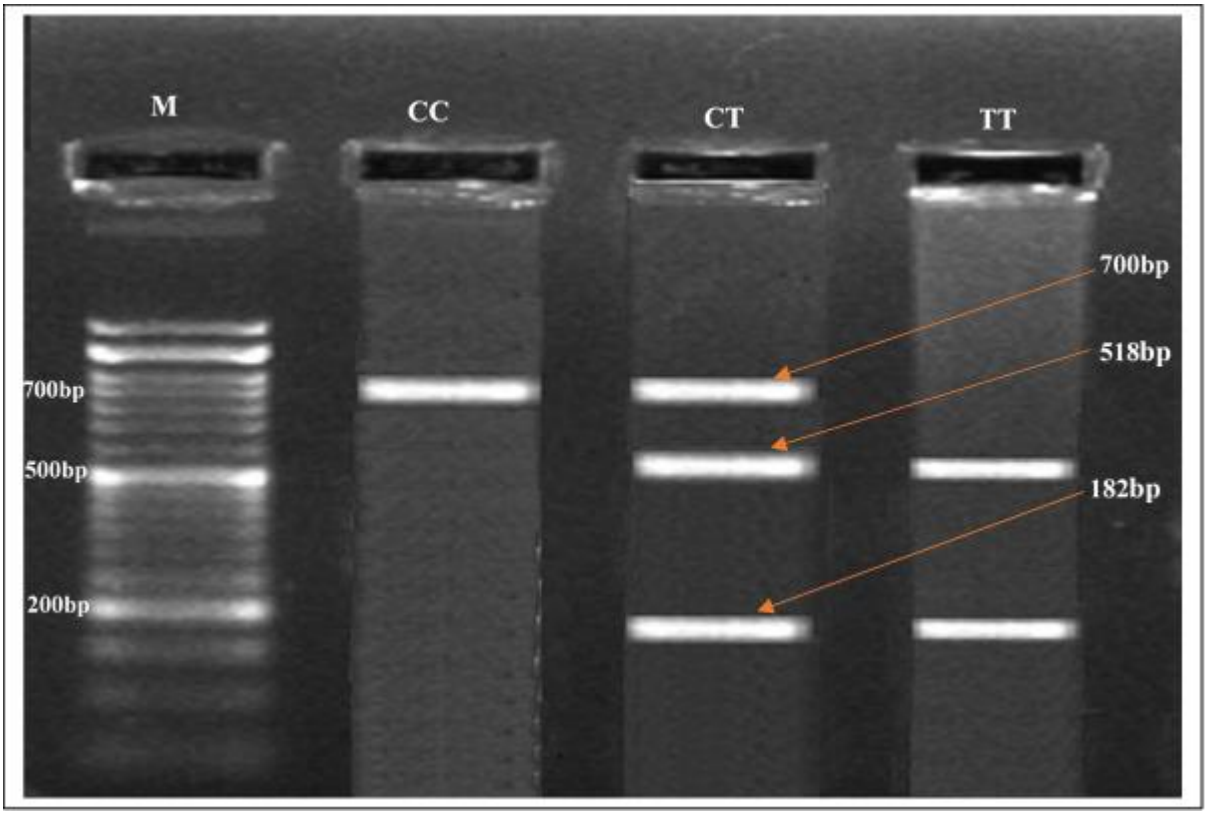

Fig. 1: Genotyping outcome for TCF7L2 gene rs7903146. M: Marker, CC genotype: 700bp, CT genotype: 700/518/182bp, and TT genotype: 518/182bp.

Both the T2DM and control groups' genotypic profiles were in Hardy-Weinberg equilibrium. The genotype and allele patterns of the rs7903146 TCF7L2 gene polymorphism in T2DM patients and healthy controls are shown in Table 2. The frequency of the "T" allele of rs7903146 (C/T) was significantly higher in T2DM (34.41%) compared with that in the controls subjects (24.35%) with the allelic odds ratio being 0.61 (95% CI, 0.43 – 0.87; p= 0.0063).

The frequencies of the CC, CT and TT genotypes of rs7903146 were 40.90%, 49.35%, 9.74% in T2DM cases and 56.49%, 38.31%, 5.19% in healthy controls respectively. In T2DM, the allele frequencies for the C and T were respectively 65.50% and 34.41% and 75.64% and 24.35% in healthy controls. Homozygous CC and heterozygous CT genotypes had a significant connection with T2DM cases compared to healthy controls (OR: 0.56; CI: 0.35-0.89; p=0.0163), and the C, T allele frequencies of the rs7903146 gene also had a significant association with T2DM cases (OR: 0.61; CI: 0.43-0.87; p=0.0063). The dominant genotype (CC vs. CT+ TT) was also examined, and we discovered a significant difference between T2DM patients and healthy controls (OR: 0.53;

CI: 0.33 - 0.83; p=0.0064). However, there was no discernible difference between T2DM cases and healthy controls for the recessive genotype (CC+CT vs. TT) (OR: 0.50; CI: 0.20 - 1.23; p=0.1351).

Table 2: Genotypes and Allele Dissemination of the TCF7L2 gene polymorphism at rs7903146 in the Case (Type 2 diabetes mellitus) and Control Groups

rs7903146 Polymorphism	Case N (%)	Controls N (%)	OR (95% CI)	p-value
Co dominant				
CC	63 (40.9%)	87 (56.49%)	1.00	-
CT	76 (49.35%)	59 (38.31%)	0.56 (0.35 – 0.89)	0.0163*
TT	15 (9.74%)	08 (5.19%)	0.38 (0.15 – 0.96)	0.0421*
Dominant				
CC	63 (40.9%)	87 (56.49%)	1.00	-
CT+ TT	91 (59.0%)	67 (43.50%)	0.53 (0.33 – 0.83)	0.0064*
Recessive				
CC +CT	139 (90.2%)	146 (94.80%)	1.00	-
TT	15 (9.74%)	08 (5.19%)	0.50 (0.20 – 1.23)	0.1351
Alleles				
C	202 (65.5%)	233 (75.64%)	1.00	-
T	106 (34.41%)	75 (24.35%)	0.61 (0.43 – 0.87)	0.0063*

Values are expressed as Number (N) and Percentage (%); OR: Odd Ratio, CI: Confidence Interval; Significant considered as P<0.05.

Clinical and biochemical characteristics of the T2DM subjects based on rs7903146 (C/T) genotypes

Comparison of metabolic traits of the T2DM participants according to their genotypes for rs7903146 (C/T) is shown in Table 3. T2DM subjects carrying the TT genotype of rs7903146 (C/T) polymorphism had higher fasting glucose level compared with the CC carriers but the statistical significance was not achieved. Similarly, the 2 hour, post parandial sugar, serum ALT level, serum AST level and serum creatinine were also higher in T2DM cases carrying TT genotype but were statistically insignificant. None of the other biochemical parameters including serum lipids, HbA1c, serum bilirubin, BUN, serum Na^+ and serum K^+ showed any significant differences among the 3 genotypes in T2DM subjects. However, the rs7903146 polymorphism significantly affected the level of GLP-1 in the blood ($p < 0.001$). When compared to the CT and TT genotypes, the serum GLP-1 level in the CC genotype was considerably greater.

Table 3: TCF7L2 gene rs7903146 genotypes- based biochemical characteristics of Cases (T2DM)

GENOTYPES→ VARIABLES ↓	CC (n=63)	CT (n=76)	TT (n=15)	p- VALUE
FBS (mg/dl)	175.26 ± 36.45	179.72 ± 29.91	187.62 ± 37.78	0.2302
PPBS (mg/dl)	235.6 ± 44.44	248.83 ± 35.33	254.32 ± 32.91	0.1598
HbA1c (%)	7.66 ± 1.39	7.65 ± 0.52	7.57 ± 0.71	0.9487
TC (mg/dl)	132.40 ± 26.50	136.96 ± 26.77	136.8 ± 21.41	0.5789
TG (mg/dl)	120.38 ± 20.72	124.04 ± 27.42	115.53 ± 17.79	0.3943
LDL (mg/dl)	80.67 ± 10.01	81.97 ± 10.65	77.66 ± 10.59	0.3257
HDL (mg/dl)	37.13 ± 10.13	37.67 ± 8.58	38.46 ± 6.88	0.8632
ALT (U/L)	30.74 ± 10.71	32.12 ± 9.68	36.57 ± 10.30	0.1398
AST (U/L)	30.24 ± 12.46	30.76 ± 9.65	32.86 ± 7.633	0.6985
ALP (U/L)	68.27 ± 21.42	73.78 ± 29.75	70.33 ± 19.24	0.4566
S.BILIRUBIN (mg/dl)	0.68 ± 0.28	0.72 ± 0.23	0.70 ± 0.25	0.6909
BUN (mg/dl)	13.54 ± 3.01	14.24 ± 3.15	13.56 ±2.73	0.3827
S. CREATININE (mg/dl)	0.83 ± 0.17	0.83 ± 0.19	0.85 ± 0.10	0.9293
Na+ (mEq/L)	137.31 ± 2.96	138.25 ± 1.76	137.46 ± 1.76	0.056
K+ ((mEq/L)	4.07 ± 0.41	4.20 ± 0.41	4.18 ± 0.54	0.2082
GLP-1 (pg/ml)	2.90 ± 0.95	1.34 ± 0.35	1.03 ± 0.87	<0.001*

Values are expressed as Mean ± Standard Deviation; *Significant considered as P<0.05. FBS: Fasting Blood Sugar, PPBS: Post-Prandial Blood Sugar, HbA1c: Glycated Haemoglobin, TC: Total Cholesterol, TG: Triglyceride, HDL: High-Density Lipoprotein, LDL: Low- Density Lipoprotein, ALT: Alanine Transaminase, AST: Aspartate Transaminase, ALP: Alkaline Phosphatase, BUN: Blood Urea Nitrogen, Na+: Sodium, K+: Potassium, GLP-1: glucagon like peptide-1

Table 4 shows multiple linear regression analysis of TCF7L2 with rs7903146 as the dependent variable. By the results it is proved that only fasting blood sugar level has a strong significant association with rs7903146 (ß= -0.322, P=0.009, CI: -0.011 to -0.002).

Table 4: Multiple linear regression analysis to show the dependence of SNP rs7903146 of TCF7L2 gene on study parameters in T2DM cases (N=154)

Coefficients[a]

Model	Unstandardized Coefficients		Standardized Coefficients	t	Sig.	95.0% Confidence Interval for B	
	B	Std. Error	Beta			Lower Bound	Upper Bound
(Constant)	-2.715	3.389		-.801	.425	-9.417	3.988
HbA1c	-.021	.064	-.032	-.325	.746	-.148	.106
FBS	-.006	.002	-.322	-2.639	**.009***	-.011	-.002
PPBS	.003	.002	.166	1.332	.185	-.001	.007
ALT	.010	.007	.160	1.356	.177	-.005	.025
AST	-.002	.007	-.041	-.348	.728	-.017	.012
ALP	.001	.002	.046	.519	.605	-.003	.005
BILIRUBIN	.091	.220	.036	.412	.681	-.345	.526
BUN	.026	.022	.122	1.156	.250	-.018	.070
S.CREATININE	-.047	.311	-.013	-.151	.880	-.662	.568
SODIUM	.025	.026	.094	.961	.338	-.027	.078
POTASSIUM	.201	.142	.135	1.419	.158	-.079	.482
TC	.003	.002	.120	1.202	.232	-.002	.008
TG	-.002	.003	-.087	-.887	.376	-.007	.003
LDL	-.005	.006	-.083	-.892	.374	-.017	.006
HDL	.007	.007	.094	1.027	.306	-.006	.020

a. Dependent Variable: GENOTYPE

* $p<0.05$ is considered significant at 95% confidence interval; HbA1c - glycated haemoglobin , FBS - Fasting Blood Sugar; Post -Prandial Blood Sugar; ALT- Alanine Transaminase, AST- Aspartate Transaminase, ALP- Alkaline Phosphatase, BUN- Blood Urea Nitrogen, TC – total cholesterol, HDL - High-Density Lipoprotein; LDL - Low-Density Lipoprotein

DISCUSSION

In the current investigation, we looked into any potential relationships between the TCF7L2 gene (SNP rs7903146) and levels of GLP-1 as well as other clinical and biochemical parameters in North Indian T2DM patients. Clinical indicators such as blood sugar, HbA1c, S.ALT, S.AST, S.Bilirubin, BUN, S.Creatinine, serum Na+, serum K+, total cholesterol, triglycerides, LDL, and HDL were considerably higher at in T2DM cases compared to healthy controls, but serum GLP-1 level was significantly lower.

According to our research, T allele of TCF7L2 rs7903146 was linked to a higher risk of T2DM, and TT genotype frequency was higher in T2DM patients. Diverse investigations in various ethnic groups produced contentious findings. According to the current research, the T allele increases the chance of

developing diabetes in the populations of Turkey and Sudan [8,9]. Conversely, in Cameroon population the T allele at rs7903146 was protective against T2DM [10]. In addition, there was no association between rs7903146 variant and T2DM in Euro-Brazilian individuals [11].

According to a recent meta-analysis by Liu et al. that included 6473 control cases and 3404 T2DM cases, the TCF7L2 gene's T allele (rs7903146) was positively associated with a higher risk of developing T2DM [12].

Our study's findings are in line with those of several studies conducted worldwide on various rs7903146 TCF7L2 gene polymorphisms and T2DM in French populations [13], Dutch [14], British [15], Germany [16], Polish [17], American [17,18], Japanese [19], and in other ethnicities [20,21,22]. These findings were supported by extensive meta-analyses that focused on various ethnicities. [22,23]. Regarding age, glycemia, HbA1c, LFT, KFT, and lipid profile, the three genotypes were not significantly different in the current study. Similar outcomes were discovered in earlier research on a Brazilian population [24].

TCF7L2 has been demonstrated to be related with lower insulin levels as opposed to higher insulin resistance [25]. This connection with T2DM, which is independent of BMI, insulin resistance, and other metabolic indicators, supports the theory that a genetic abnormality in TCF7L2 causes decreased insulin secretion [22]. Unfortunately, our cohort's insulin levels were not examined. The phenotypic expression of the variant may differ in the examined groups because interactions with other distinct genetic or environmental factors may modify the susceptibility brought on by the polymorphism.

The very modest sample size and number of SNPs examined in the current study may initially appear to be its principal limitations. Large-scale research is therefore necessary to confirm the genotype-phenotype relationship found in the Indian population. Furthermore, it would be desirable to investigate any other TCF7L2 gene SNPs in our sample since this may reveal additional associations with the risk of acquiring diabetes and its complications. Using whole-genome or whole-exome sequencing will provide you a thorough understanding of your vulnerability to T2DM development.

CONCLUSION

In our study, TCF7L2 was related to T2DM in the studied North Indian population and both T allele and TT genotype of rs7903146 (C/T) was associated with high risk of T2DM. There was significant association of CC and CT genotype as well as CC and TT genotype. Also the C and T allele frequencies of the rs7903146 are significantly associated with T2DM cases as compared to healthy controls. It was observed that CC genotype of rs7903146 has significant impact on circulatory GLP-1 levels in cases as compared to controls.

CONFLICT OF INTEREST

Authors declare that there is no conflict of interest.

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