Journal of Scientific and Technical Research
11 (1) June 2021, 19-28
http://www.publishingindia.com/jstr

Genetic Association of a Polymorphic Variant (rs7903146) in TCF7L2 Gene by Allele-Specific Genotyping to Predict the Susceptibility of T2DM

Monika Sharma[1], Arif Ahmad[2], Arti Sharma[3], Jamal Ahmad[4], Badaruddoza[5], Riaz Ahmad[1] and Iqbal Parwez[1,6*]

[1]Department of Zoology, Aligarh Muslim University, Aligarh, Uttar Pradesh, India
[2]School of Sciences (Zoology), Maulana Azad Urdu University, Hyderabad, India
[3]Department of Statistics and Operations Research, Aligarh Muslim University, Aligarh, Uttar Pradesh, India
[4]Former Director, Rajiv Gandhi Centre for Diabetes and Endocrinology, Faculty of Medicine, J. N. Medical College, Aligarh Muslim University, Aligarh, Uttar Pradesh, India
[5]Department of Human Genetics, Guru Nanak Dev University (GNDU), Amritsar, Punjab, India
[6]School of Biosciences and Biotechnology, Baba Ghulam Shah Badshah University, Rajouri, Jammu & Kashmir, India
*Corresponding Author: iparwez2002@gmail.com

Abstract: In type 2 diabetes mellitus (T2DM), transcription factor 7- like 2 (TCF7L2) gene has been linked its susceptibility in multiple ethnicities. This case-control study investigates the possible role of TCF7L2 variant; rs7903146 (C/T) along with anthropometric data and clinical profiles in the North Indian population. TCF7L2 polymorphism was genotyped by allele-specific PCR assay. Odds ratio (OR) and 95% Confidence Intervals were used to assess the strength of genetic association. A total of 500 human subjects consisting of 250 T2DM patients together with 250 normoglycemic controls from the region of North India. Out of total T2DM subjects, 64.4% had a family history and 74% were reported to be hypertensive. Most of the anthropometric markers showed a statistically significant ($p<0.0001$) association with T2DM when compared to normoglycemic controls, specifically in fasting glucose, postprandial glucose, and HbA1c. Further analysis indicates that recessive genetic model of association established that the unadjusted odds ratio of risk homozygous (TT) subjects was 2.6 fold higher (OR=2.63; 95% CI=1.27-5.43) compared to wild homozygous (CC) subjects. Simultaneously, when adjusted with age, sex, and BMI, this risk was increased up to 3.4 fold (OR=3.46; 95% CI=1.63-7.34). Risk alleles (TT subjects) were also found to be statistically significant in BMI and blood sugar parameters. This SNP was also showed significant statistical association with T2DM under dominant and co-dominant models of logistic regression even after adjusting the covariates. This study exposed the positive genetic association of the risk variant rs7903146 (T) SNP of TCF7L2 in T2DM subjects of the North India.

Keywords: rs7903146, Single nucleotide polymorphism, TCF7L2, Type 2 Diabetes Mellitus (T2DM).

I. Introduction

Type 2 Diabetes Mellitus (T2DM) is a complex heterogeneous group of metabolic disorders influenced by the interactions among genetic/epigenetic, behavioral, and socioeconomic factors, i.e., genes with the environment (Cuschieri, 2019). In 2020, the population status of T2DM identified by the International Diabetes Federation (IDF) reported 463 million people were affected, 88 million in the region of Southeast Asia (SEA), of which 77 million is from India only constituting the prevalence of 8.9% amongst the total population (Saeedi *et al.,* 2019). In India, the prevalence scale of T2DM showed broad regional disparities such as 5.4% in North, 12.3-15.5% in South, and 12.3-16.8% in Central India (Gupta & Misra, 2007). Indians have recorded a little higher prevalence of T2DM than Europeans, as it is also influenced by "Asian Indian Phenotype" rather than obesity and BMI values (Mohan & Deepa, 2006).

Several genes (ENPP-1, KCNJ11, PPARG, KCNQ1, FTO, etc.) were reported to play an important role in the etiology of T2DM (Deeb *et al.,* 1998; Sanghera *et al.,* 2008; Tsai *et al.,* 2010; Wang *et al*., 2011; Yazdi *et al.,* 2020). One of the most important genes i.e. transcription factor 7- like 2 (TCF7L2) has a role in the insulin signaling pathway. It is highly replicated and is the most significant gene for T2DM. TCF7L2 gene spans 215.9 kb region on chromosome 10q25.3 (Grant *et al.,* 2006). The human TCF7L2 gene formerly referred to as "TCF-4" is a

transcription factor articulated with the WNT signaling pathway and functions as a nuclear receptor for CTNNB1 (β-catenin). TCF7L2 creates a heterodimer with β-catenin in the Wnt signaling pathway which is involved in cell proliferation and embryonic development, where it influences the production of pancreas/islets, β-cell growth, β-cell mass, and β-cell function (Ip *et al.*, 2012). It induces the expression of numerous genes, including insulin gene, intestinal proglucagon, glucagon-like peptide-1 (GLP-1), and glucagon-like peptide-2 (GLP-2), insulin conversion factors, and genes critical for β-cell proliferation (Rulifson *et al.*, 2007). Additionally, TCF7L2 (rs7903146) is predominantly involved in incretin secretion/sensitivity (Færch *et al.*, 2013). The SNP (rs7903146; C/T) in the TCF7L2 gene was reported to have differential expression in human pancreatic islets. It is located in the intronic region with no explicit mechanism by which it alters the TCF7L2 function (Helgason *et al.*, 2007). It has an inverse relationship with glucose-stimulated insulin secretion. It is also involved in the mechanism of insulin secretion in numerous studies and the presence of SNP is assumed for its impairment (Munoz *et al.*, 2006; Schafer *et al.*, 2007; Kirchhoff *et al.*, 2008). Common variants found in the region of TCF7L2 could be the reason for reduced insulin secretion rather than insulin resistance (Gloyn *et al.*, 2003). The TCF7L2 gene has shown a consistent association with T2DM among several populations in various ethnicities (Grant *et al.*, 2006; Groves *et al.*, 2006; Chandak *et al.*, 2007; Lyssenko *et al.,* 2007; Turki *et al.,* 2013; Badaruddoza *et al.,* 2015). The earlier study on the TCF7L2 gene is on G/T (rs1225572) variant and has a significant association with T2DM with a recessive model of action in a small group of the North Indian Punjabi population (Badaruddoza *et al.,* 2015). However, it is strongly desirable to further expand the study to a larger segment of the North Indian population to capture a more comprehensive view of this phenomenon. This study, therefore, examined in detail the genetic association of C/T (rs7903146) variant of TCF7L2 gene with anthropometric data and clinicopathological variables of T2DM in a larger segment of the North Indian population.

II. Materials and Methods

A. Subjects and Study Design

The present work is based on a case-control study to assess the genetic association of SNP rs7903146 of TCF7L2 in cases (N=250) and controls (N=250) of the North Indian population. The sample sizes were calculated by "CaTs power calculator" before the start of the study to get more than 80% Power to detect statistically significant ($p<0.05$) difference between T2DM-Normoglycemic controls either clinically or biologically. The study was approved by the Bio-Ethical Committee *(Study No: ECR/419/INST/UP/2013),* Faculty of Medicine, Jawaharlal Nehru Medical College, Aligarh Muslim University (JNMC, AMU), Aligarh, India.

B. Enrolment of T2DM Subjects and Normoglycemic Controls (NCs)

A total of 500 unrelated, age and sex-matched samples with 250 T2DM patients and 250 Normoglycemic Control (NC) were selected from the discrete population of the Northern part of India for the clinical and genetic analysis of TCF7L2. As this is a retrospective study, all the clinical data were collected from the patient's case history, visiting the OPD of Rajiv Gandhi Centre for Diabetes & Endocrinology, JNMC, AMU regularly. Normoglycemic control samples were collected voluntarily. Written consent was obtained from each subject after explaining the research study. Collection of peripheral blood was also done from each participant of the study. Diagnosis of T2DM is based on criteria of the American Diabetes Association, 2017. Those with fasting glucose (FG) value of more than 126 mg/dl (7.0 mmol/L) and Postprandial Glucose (PPG) value of more than 200 mg/dl (11.1 mmol/L) are cases, and those with no family history of T2DM with FG and PPG value less than above values served as normoglycemic control.

C. Anthropometric and Clinical Markers of Normoglycemic Controls (NCs) and T2DM Subjects

Relevant information of phenotype and covariates, demographic data such as age, sex, religion, and other relevant symptoms and blood parameters were recorded. Basic features included current age and age of onset of T2DM. Anthropometric data include height (cm), weight (Kg), waist circumference (cm), hip circumference (cm), Systolic Blood Pressure (SBP) & Diastolic Blood Pressure (DBP) (mmHg), and pulse rate (per min). Derived parameters included BMI (Kg/m^2), Pulse Pressure (mmHg), Mean Arterial Pressure (mmHg), Waist to Hip ratio (WHR). Clinically defined parameters such as Fasting and Postprandial glucose (mg/dL) along with glycosylated Hemoglobin (HbA1c) (%)/ (mmol/mol) were also recorded.

D. DNA Extraction and Gene Polymorphism

The peripheral blood samples (5 ml) were collected in EDTA-anticoagulated and serum collection tubes. Genomic DNA was extracted from whole blood using the salting-out method (Miller *et al.,* 1988) with minor laboratory modifications. Allele-Specific PCR (AS-PCR) (Ugozzoli & Wallace, 1991) was used to detect transcription factor 7- like 2 (TCF7L2) gene polymorphism. Genotyping the T allele (rs7903146) by AS-PCR required a set of three primers i.e., two forward primers contained a mismatch at their 3'end in such a way that each specified for one of the two nucleotides of the polymorphism and a common reverse primer that did not contain the mismatches, present downstream of the polymorphic location. The allele-specific primers were selected on the basis of previous study (Dutra *et al.,* 2008), forward primer 1 (C Allele: gaacaattagagagctaagcacttttagaaac), forward primer 2 (T Allele: gaacaattagagagctaagcacttttagagat), and common reverse primer (C/T Allele; agatgaaatgtagcagtgaagtgc). Two

parallel PCR reactions were run to genotyped the rs7903146 polymorphism of TCF7L2; one with C Allele forward primer, one with T allele forward primer along with a common C/T allele reverse primer. Each 25 µl PCR reaction mixture contain 10X PCR buffer (100 mM Tris, 500 mM KCl,) with 2.5 mM $MgCl_2$, 10 mM dNTPs, 1.5U Taq DNA polymerase (Thermo Fisher Scientific), 10 pmoles/µl each primer (Sigma Aldrich), and 50-100 ng template DNA. The PCR was carried on a Thermocycler (PEQSTAR 2X Gradient) under the following conditions; "Heat Lid 110 ^{0}C, Initial Denaturation 94 ^{0}C for 5 min, Denaturation 94 ^{0}C for 1 min, Annealing Temperature 48 ^{0}C for 45 sec, Primer Extension 72 ^{0}C for 1 min, and Final Extension 72 ^{0}C for 5 min". The PCR product (205 bp) was electrophoreses on 2% agarose gel and visualized in a gel documentation system (Bio-Rad).

E. Statistical Analysis

All the statistical analysis for SNP was performed by using R (version 4.0.2) software utilizing "Genetics/SNP Packages". All the data of continuous variables were expressed in the form of mean ± SD. Comparison of means between T2DM subjects with Normoglycemic controls (NC) was measured by Student's t-test (two-tailed). ANOVA was used to compare among genotypes of T2DM and controls separately. Comparison of genotype and allele frequencies of TCF7L2 variant of T2DM subjects with Normoglycemic controls (NC) using Pearson Chi-square (χ^2) statistics. Hardy Weinberg Equilibrium was also estimated by χ^2 goodness of fit test. The Multiple logistic Regression (MLR) analysis was used to study the genetic association by calculating Odds Ratios (ORs), 95% Confidence Intervals (CIs), and p-value for TCF7L2 polymorphism before and after adjusting for potential covariates, i.e., age, sex, and BMI. The $p<0.05$ were considered statistically significant for all statistical procedures.

III. Results

The present study analyzed the anthropometric data, clinical profiles, and SNP (rs7903146; C/T) analysis of T2DM along with Normoglycemic Controls (NCs). The mean age of T2DM and NCs were 49.88 ± 9.53 and 49.23 ± 10.56, respectively. The mean age of onset of T2DM calculated in our study is 46.52 ± 9.11. The T2DM/NCs subjects contain 55.6% females/53.2% females and 44.4% males/46.8% males. Majority of the T2DM subjects i.e. 64.4% had a family history, and 74% are hypertensive.

The primary baseline data and clinical parameters of both the groups were expressed in the form of mean ± SD in Table I. Of the t-test statistics, no significant difference was observed for age, weight, and age of onset of the study subjects. The rest of the parameters showed statistically significant ($p<0.0001$) variations in T2DM subjects compared to Normoglycemic controls. They were reported as highly significantly different in Fasting Glucose (mg/dL), PP Glucose (mg/dL), and HbA1c (%) in T2DM / NCs; i.e. 166.29 ± 60.05 / 90.12 ± 13.11, 246.28 ± 78.96 / 138.49 ± 16.35, and 8.6 ± 1.96 / 5.8 ± 0.96 respectively, which is statistically ($p<0.0001$) significant. Other parameters were also recorded variable data in T2DM/NCs as represented by BMI (26.14±4.53/23.66±2.84; $p<0.0001$), waist circumference (99.19±11.23/96.43±7.36; $p<0.001$), hip circumference (102.12±9.83/104.66±6.43; $p<0.0007$), waist to hip ratio (0.97±0.06/0.92±0.05; $p<0.0001$). A significant nominal ($p<0.001$) difference was found in the case of waist circumference. The considerable difference was also observed in blood pressure of T2DM/NCs, especially for systolic one (134.12 ± 20.18/ 120.99 ± 12.03; $p<0.0001$) rather than diastolic (83.08 ± 10.52/80.68 ± 7.46; $p<0.003$). Few parameters represented only considerable higher values in patients which, however, were not statistically significant i.e., Pulse pressure (difference of SBP and DBP) was reportedly higher in T2DM (83.47 ± 9.66) compared to controls (78.17 ± 10.27), Mean Arterial Pressure value in T2DM patients (99.78 ± 12.8) was also higher than controls (94.90 ± 16.41), pulse rate of T2DM is significantly higher in T2DM (83.47 ± 9.66) and Normoglycemic Controls (78.17 ± 10.27).

Table I: Anthropometric and Clinical Parameters of T2DM Subjects and Normoglycemic Controls

Anthropometric and Clinical Characteristics	*T2DM Patients (n= 250)*	*Normoglycemic Controls (NC) (n= 250)*
Age (years)	49.88±9.537	49.23±10.56
Sex (M/F)	111/139	117/133
Age of onset (years)	46.52±9.11	NA
Height (cm)	158.70±9.48	164.07±10.00
Weight (Kg)	65.72±11.89	63.92±10.77
BMI (Kg/m^2)	26.14±4.53*	23.66±2.84
Waist Circumference (cm)	99.19±11.23	96.43±7.36
Hip Circumference (cm)	102.12±9.83	104.66±6.43
WHR	0.97±0.06*	0.92±0.05
SBP (mmHg)	134.12±20.18*	120.99±12.03
DBP (mmHg)	83.08±10.52	80.68±7.46
Pulse Pressure (PP) (mmHg)	51.26±15.72*	40.35±8.12
Mean Arterial Pressure (MAP) (mmHg)	99.78±12.8	94.90±16.41
Pulse Rate (PR) (per min)	83.47±9.66*	78.17±10.27
Fasting Glucose (mg/dL)	166.29±60.05*	90.12±13.11
PP Glucose (mg/dL)	246.28±78.96*	138.49±16.35
HbA1c (%)	8.60±1.96*	5.87±0.96

All continuous variables are presented in mean ± SD. BMI; Body Mass Index, SBP; Systolic Blood Pressure, DBP; Diastolic Blood Pressure, HbA1c; Glycosylated hemoglobin. *Significant at $p<0.0001$; t -test was used to compare the means of T2DM subjects and Normoglycemic controls.

The genotypic association of TCF7L2 (rs7903146) variant and quantitative markers related to the susceptibility of T2DM are presented in Table II. In T2DM subjects and Normoglycemic individuals, all the traits were tested based on homozygous allele (CC) for SNP compared to heterozygous (CT) and mutant homozygous (TT), assuming a dominant effect. In the case of T2DM, BMI values were higher (p=0.02) in CT & TT genotypes compared to CC. Furthermore, the potential influence of this variant (T allele) of TCF7L2 were also recorded on fasting (p=0.04) & postprandial blood sugar (p=0.01) along with HbA1c values (p= 0.03).

Table II: The Association of Genotypes of TCF7L2 (rs 7903146) with Anthropometric and Clinical Parameters

Anthropometric and Clinical Characteristics	*T2DM Patients*			*Normoglycemic Controls*		
	CC (133)	*CT (90)*	*TT (27)*	*CC (166)*	*CT (73)*	*TT (11)*
Age (years)	50.5 ±9.89	48.8±8.70	50.2±10.5	49.1±10.9	49.3±10.1	50.4±8.98
Age of onset (years)	47.3±9.39	45.3±8.44	46.7±9.68	-	-	-
Height (cm)	158±9.84	159±9.34	160±8.03	165±10.1	162±9.57	169±8.56
Weight (Kg)	66.4±12.3	66±11.8	61.4±9.69	63.8±10.6	64±11.3	65.2±10.4
BMI (Kg/m^2)	24.3±4.79	26±4.28	26.6±3.45*	23.4±2.76	24.3±3.02	22.7±2.44
Waist Circumference (cm)	99.3±11.7	100±11.2	95.2±8.06	96.1±7.61	96.7±6.82	99.1±6.92
Hip Circumference (cm)	102±9.71	103±10.5	98.8±7.60	104±6.69	105±5.88	107±6.00
WHR	0.97±0.06	0.97±0.06	0.96±0.04	0.92±0.05	0.92±0.06	0.92±0.03
SBP (mmHg)	132±20.6	132±18.4	136±23.7	121±11.7	122±12.8	118±11.8
DBP (mmHg)	83.4±11	82.4±9.45	83.4±11.6	80.2±7.46	81.8±7.51	79.5±6.83
Pulse Pressure (PP) (mmHg)	52.9±16.9	49.7±13.6	48.4±15.9	40.5±7.95	40.3±8.19	38.3±10.5
Mean Arterial Pressure (MAP) (mmHg)	101±13.2	98.5±11.6	99.5±14.8	93.7±8.29	98.1±27.4	92.3±7.07
Pulse Rate (PR) (per min)	83.9±9.91	82.8±9.36	83.5±9.61	77.8±10.2	79±10.5	78.6±9.44
Fasting Glucose (mg/dL)	161±60.8	165±60.2	173±56.8*	90.1±13.1	90.8±12.7	85.4±16.4
PP Glucose (mg/dL)	238±75.3	244±85.1	258±76.3*	133±17.7	138±13.9	139±8.20
HbA1c (%)	8.45±1.88	8.71±2.02	9.02±2.17*	5.80±0.95	5.8±0.98	6.06±0.82

All continuous variables are presented in mean ± SD. BMI; Body Mass Index, SBP; Systolic Blood Pressure, DBP; Diastolic Blood Pressure, HbA1c; Glycosylated hemoglobin.* Significance at p<0.05; ANOVA was used to compare the quantitative means across genotypes.

Association of rs7903146 Polymorphism of TCF7L2 with T2DM

Based on the risk allele (T) of rs7903146 of TCF7L2, T2DM subjects showed substantial differences with Normoglycemic Controls (NCs). Testing genetic association between SNP and T2DM implemented with multiple logistic regression includes age, sex, and BMI as covariates. The rs7903146 has two alleles: C and T. The presence or absence of a 205 bp band in parallel electrophoresis of C/T indicated the type of genotype (CC/CT/TT) present in an individual. The genotypes of T2DM - Normoglycemic controls are in Hardy Weinberg Equilibrium (HWE), and the detailed data are shown in Table III. The percentage distributions of genotypes (CC, CT & TT) are shown in Fig. 1. This data confirmed the minor allele (T) frequency was significantly higher in T2DM subjects (28.8%) in comparison to controls (19%). Similarly, TT's genotypic frequency was also significantly higher in T2DM compared to normoglycemic controls (10.8% vs. 4.4%).

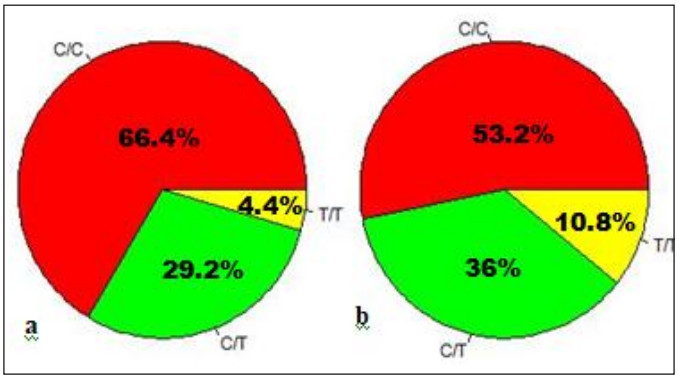

Fig. 1: Percent ratio among genotypes showing C/C; homozygous wild type, C/T; heterozygous, T/T; risk homozygous genotype distribution in normoglycemic controls (a) and T2DM patients (b).

Multivariate Logistic Regression analysis of rs7903146 produced statistically significant results for the minor allele

genotype (TT) in comparison to reference allele genotype (CC) and presented the Odds Ratio (ORs) of 2.63 with 95% Confidence Intervals (95% CIs; 1.27 to 5.43). The odds ratio adjusted for covariates, i.e., age, sex, and BMI, also represented with little higher risk (OR - 3.46; 95% CIs - 1.63 to 7.34). SNP (rs7903146) is significantly associated with T2DM in unadjusted/adjusted studies under the co-dominant model (ORs -3.06/3.97), dominant model (ORs-1.74/1.79). All the p-values were less than 0.05 and hence considered statistically significant. Table III summarizes the details of genetic association studies of rs7903146 with T2DM under multiple logistic regression models. Fig. 2 showed the frequency of all the three genotypes i.e. CC, CT, and TT resolved on agarose gel representing the presence or absence of C/T allele in T2DM and Normoglycemic controls.

Table III: Genotypic, Allelic Frequencies, and Estimates of Relative Risk under Different Genetic Models of Association for TCF7L2 (rs7903146) Polymorphism in T2DM Patients and Normoglycemic Control

Gene (SNP)	*Genotype (C/T)*		*Co-Dominant Model OR (95% CIs) p-Value*	*Dominant Model OR (95% CIs) p-Value*	*Recessive Model OR (95% CIs) p-Value*
	Normoglycemic Controls (NC)	*T2DM Patients*			
Unadjusted Odds Ratio					
TCF7L2 (rs7903146)	N (%)	N (%)	CC vs CT, TT	CC vs CT/TT	CC/CT vs TT
CC	166 (66.4)	133 (53.2)	1 (Reference)		
CT	73 (29.2)	90 (36)	1.54 (1.05-2.26) 2.0×10^{-3}	1.74 (1.21-2.50) 2.5×10^{-3}	2.63 (1.27-5.43) 6.1×10^{-3}
TT	11 (4.4)	27 (10.8)	3.06 (1.47-6.40) 2.0×10^{-3}		
Adjusted Odds Ratio with Age, Sex, and BMI					
CC	166 (66.4)	133 (53.2)	1 (Reference)		
CT	73 (29.2)	90 (36)	1.49 (1.00-2.24) 4×10^{-4}	1.79 (1.22-2.62) 2.5×10^{-3}	3.46 (1.63-7.34) 6×10^{-4}
TT	11 (4.4)	27 (10.8)	3.97 (1.85-8.55) 4×10^{-4}		
C Allele	0.81	0.72	1.65 (1.24 - 2.19) 1.0×10^{-3}		
T Allele	0.19	0.28			
Chi-Square (p-Value)	0.6587 (0.417)	3.7327 (0.043)	SNP; Single Nucleotide Polymorphism, HWE; Hardy Weinberg Equilibrium, CC; homozygous genotype, CT; Heterozygous genotype, TT; Mutant genotype, CIs; Confidence intervals, OR; Odds ratio. # Most common genotype (CC) in the population is taking as reference for Statistical procedures (p value < 0.05) in genetic association studies.		

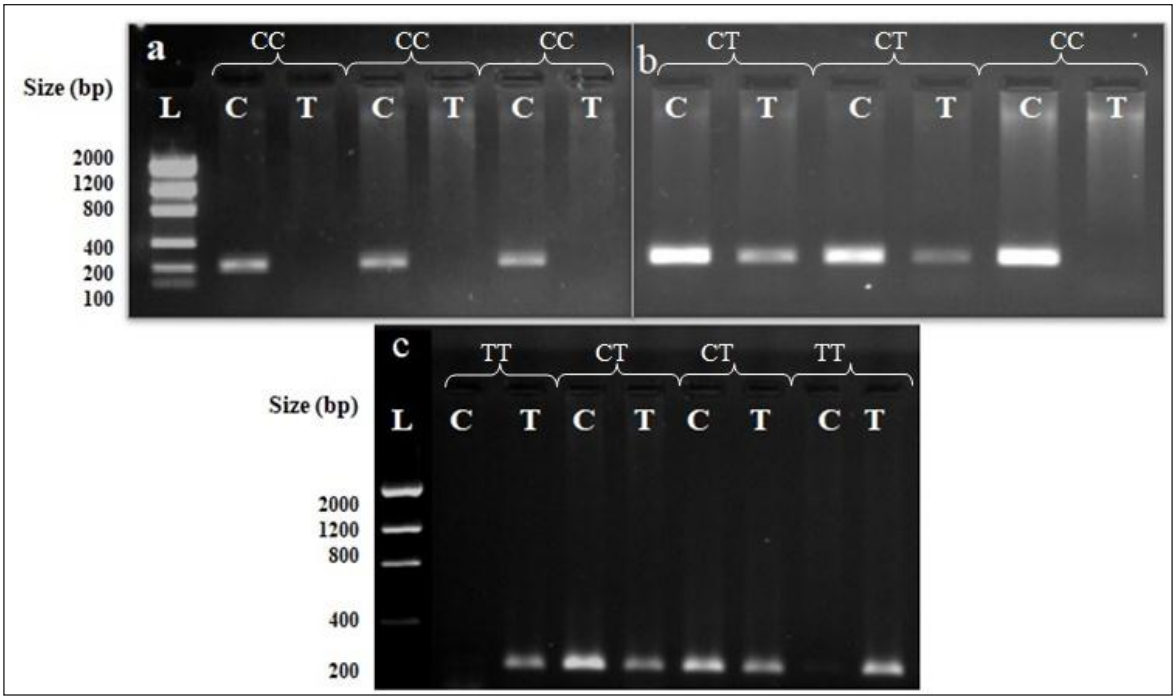

Fig. 2: PCR products resolved on agarose gels showing genotyping of rs7903146 (C/T) by allele-specific PCR assay. For each sample two parallel PCR reactions were designed, one with C allele primer, the other with T allele primers along with common reverse primer, and run in parallel on agarose gel (2%). A 205 bp band in Normoglycemic controls (a) and T2DM patients (b, c) represented all three genotypes (CC/CT/TT). In agarose gel, the first lane with standard 2 Kb DNA ladder (L) and other lanes with parallel C/T indicated for the product of similar individual amplified from PCR reactions.

IV. Discussion

T2DM is one of the largest global health emergencies in the 21st century. All the genetic and environmental factors do not affect every individual equally but paved the way differently by highlighting genetic susceptibility to the manifestation of T2DM. Initial studies (Grant *et al*., 2006; Helgason *et al*., 2007) demonstrated that the non-coding variant (rs7903146), single nucleotide polymorphism of TCF7L2 remains the most probable candidate variant to detect the genetic association. In the present study, we have selected the North Indian population to study the TCF7L2 polymorphism for this particular SNP. TCF7L2 (rs7903146) is one of the eight well-established gene variants of TCF7L2 which have already been reported with a larger effect size in the Indian population than Europeans (Chauhan *et al.*, 2010). We determined the genotype and allele frequencies of TCF7L2 (rs7903146) polymorphism and the potential association of quantitative traits and SNP with T2DM.

Even though numerous genes i.e. PPARG, KCNJ11, ENPP-1, IRS 1, FTO HHEX/IDE, IGF2BP2, SLC30A8, KCNQ1, etc. showed the association with T2DM but TCF7L2 gene demonstrated the strongest association till now. The present study established that parameters like age, height, weight, BMI, WC, HC, WHR, SBP, DBP, pulse pressure (SBP – DBP), pulse rate, mean arterial pressure, fasting and postprandial blood sugar, and HbA1c showed statistical ($P<0.0001$) significance. BMI, fasting and postprandial blood sugar was also statistically significantly different in T2DM subjects with simultaneous higher values of HbA1c in TT genotype Individuals. In 2016, one of the co-authors of this study already described the positive correlation of HbA1c with fasting glucose ($R^2 = 0.381$) and other blood & serum markers in T2DM patients of virtually similar ethnic origin (Doddigarla *et al.*, 2016). Similarly, in our study, fasting/PP glucose and HbA1c values are significantly higher (TT variant) in T2DM subjects than normoglycemic controls. A recent study also corroborated the concept that the rs7903146 variant of TCF7L2 showed a statistically significant linear interaction between BMI/WC along with blood glucose levels (Li *et al.*, 2019). This genetic variant in a recent meta-analysis also confirmed the association with gestation diabetes in Indian women (Khan *et al.*, 2019).

The current study suggested a positive genetic association of TCF7L2 (rs7903146) between the risk allele (T) and T2DM under the three genetic models i.e. Co-dominant, Dominant and Recessive. For rs7903146, SNP risk homozygous (TT) subjects had a risk of 2.6 fold. However, when adjusted with potential covariates (age, sex, and BMI), this risk gets increased up to 3.4 fold. These observations suggest that age, gender, and BMI also played a substantial role in developing T2DM. This particular SNP recorded both positive and negative associations in diverse population groups (Peng *et al*., 2013). It is evident from earlier studies that the higher prevalence of the T allele of rs7903146

increased the susceptibility of T2DM. Multiple National and International groups corroborated their research regarding the vulnerability of this and other gene variants of TCF7L2 with T2DM in the various populations (Humphries *et al.,* 2006; Bodhini *et al.,* 2007; Cauchi *et al.*, 2007; Chandak *et al.*, 2007; Chang *et al.*, 2007; Sladek *et al.,* 2007; Zeggini *et al.,* 2007). Similar to this study, a recent study on Egyptians showed a 2.3 fold higher risk of T2DM in TT individuals (El-Lebedy & Ashmway 2016); and 3.34 fold in Palestinians (Ereqat *et al.,* 2010). Combining the risk variants in TCF7L2 with those in other genes like PPARG and KCNJ11 increased the Odd Ratio (OR) up to 3 (Ridderstrale & Groop, 2009).

Our case-control study supported the positive association between TT genotype and T2DM. The minor allele (T) frequency recorded in the current study was 28% for T2DM subjects and 19% in normoglycemic controls. The T allele frequency of this data is more closely related to Asian Indians (33%) (Humphries *et al.*, 2006). Though T allele frequency of T2DM patients may also vary in other populations like 5.3% in Japanese (Hayashi *et al.*, 2007), 34.5% in Danish (Helgason *et al.*, 2007), 36% in UK resident South Asian (Rees *et al.*, 2008), 40% in Emirati subjects (Saadi *et al.*, 2008), 47.3% in Persians (Palizban *et al.*, 2012), and the highest among all is 49.7% in Palestinians (Ereqat *et al.*, 2010). Ethnic difference is one of the significant reasons for genetic heterogeneity. A very recent meta-analysis combined data from the year 2000-2017 to determine the relation of rs7903146 gene variant of TCF7L2 and showed significant association with disease progression in almost every ethnic population (Ding *et al.*, 2018).

However, variable results of genetic association emerged due to ethnic differences, sample size, study design, and gene-gene or gene-environmental interactions. Ethnically, the strong genetic association of this particular susceptible gene (SNP) along with the other biological predictors (BMI, WHR) could have also helped in predicting the risk of developing T2DM at an earlier age. The present study emphasizes the equal association of the baseline parameters and allelic variations as potential risk factors for T2DM. The study suggested a positive genetic association with the risk of the variant (rs7903146; T allele) of TCF7L2 and showed its susceptibility with T2DM subjects in the North Indian population.

Acknowledgments

The authors acknowledge the help rendered by the Chairman, Department of Zoology, Faculty of Life Sciences, Aligarh Muslim University, Aligarh, Uttar Pradesh (UP), India during this study. The authors are also grateful to the Vice-Chancellor, Aligarh Muslim University, Aligarh, India for facilitating the availability of the laboratory facilities. I also wanted to acknowledge the funding agencies as this work was financially supported under the program 'Promotion of University Research and Scientific Excellence (PURSE) Programme - Phase - II (F.6-5-EP/DS/148)', of Department of Science & Technology, New Delhi, India and University Grants Commission, New Delhi, India Non-NET fellowship to MS (D.No.1906/FLS).

References

[1] B. Badaruddoza, B. Barna, K. Matharoo, and A. J. S. Bhanwer, "A case-control association study of K121Q (rs 1044498) and G/T (rs 1225572) variants in ENPP1 and TCF7L2 genes with type 2 diabetes mellitus in north Indian Punjabi population," *Int J Diabetes Dev Ctries*, vol. 35, no. 4, pp. 546-553, 2015. Available: https://doi.org/10.1007/s13410-015-0337-9

[2] D. Bodhini, V. Radha, M. Dhar, N. Narayani, and V. Mohan, "The rs12255372(G/T) and rs7903146(C/T) polymorphisms of the TCF7L2 gene are associated with type 2 diabetes mellitus in Asian Indians," *Metab Clin Exp.,* vol. 56, no. 9, pp. 1174-1178, 2007. Available: https://doi.org/10.1016/j.metabol.2007.04.012

[3] S. Cauchi, Y. El Achhab, H. Choquet, C. Dina, F. Krempler, R. Weitgasser, C. Nejjari, W. Patsch, M. Chikri, D. Meyre, and P. Froguel, "TCF7L2 is reproducibly associated with type 2 diabetes in various ethnic groups: A global meta-analysis," *J Mol Med*., vol. 85, no. 7, pp. 777-782, 2007. Available: https://doi.org/10.1007/s00109-007-0203-4

[4] G. R. Chandak, C. S. Janipalli, S. Bhaskar, S. R. Kulkarni, P. Mohankrishna, A. T. Hattersley et al., "Common variants in the TCF7L2 gene are strongly associated with type 2 diabetes mellitus in the Indian population," *Diabetologia*, vol. 50, no. 1, pp. 63-67, 2007. Available: https://doi.org/10.1007/s00125-006-0502-2

[5] Y. C. Chang, T. J. Chang, Y. D. Jiang, S. S. Kuo, K. C. Lee, K. C. Chiu, and L. M. Chuang, "Association study of the genetic polymorphisms of the transcription factor 7-like 2 (TCF7L2) gene and type 2 diabetes in the Chinese population," *Diabetes*, vol. 56, no. 10, pp. 2631-2637, 2007. Available: https://doi.org/10.2337/db07-0421

[6] G. Chauhan, C. J. Spurgeon, R. Tabassum, S. Bhaskar, S. R. Kulkarni, A. Mahajan et al., "The risk of type 2 diabetes in 5,164 Indians," *Diabetes*, vol. 59, no. 8, pp. 2068-2074, 2010. Available: https://doi.org/10.2337/db09-1386.G.C.

[7] S. Cuschieri, "The genetic side of type 2 diabetes - A review," *Diabetes Metab Syndr,* vol. 13, no. 4, pp. 2503-2506, 2019. Available: https://doi.org/10.1016/j.dsx.2019.07.012

[8] S. S. Deeb, L. Fajas, M. Nemoto, J. Pihlajamäki, L. Mykkänen, J. Kuusisto et al., "A Pro12Ala substitution in PPARγ2 associated with decreased receptor activity, lower body mass index and improved insulin sensitivity,"

Nat Genet, vol. 20, no. 3, pp. 284-287, 1998. Available: https://doi.org/10.1038/3099

[9] W. Ding, L. Xu, L. Zhang, Z. Han, Q. Jiang, Z. Wang, and S. Jin, "Meta-analysis of association between TCF7L2 polymorphism rs7903146 and type 2 diabetes mellitus," *BMC Med Genet*, vol. 19, no. 1, pp. 1-12, 2018. Available: https://doi.org/10.1186/s12881-018-0553-5

[10] Z. Doddigarla, I. Parwez, and J. Ahmad, "A hospital based study on correlation between hyperglycemia, glycated hemoglobin, lipid and oxidative stress variables in type 2 diabetes mellitus subjects: A cross sectional analysis," *World J Pharm Res*, vol. 5, no. 8, pp. 729-736, 2016. Available: https://doi.org/10.20959/wjpr20168-6701

[11] L. A. S. Dutra, P. G. G. Costa, L. F. R. Velasco, A. A. Amato, and G. B. Barra, "Allele-specific PCR assay to genotype SNP rs7903146 in TCF7L2 gene for rapid screening of diabetes susceptibility," *Arq Bras Endocrinol Metabol*, vol. 52, no. 8, pp. 1362-1366, 2008. Available: https://doi.org/10.1590/S0004-27302008000800026

[12] D. El-Lebedy, and I. Ashmawy, "Common variants in TCF7L2 and CDKAL1 genes and risk of type 2 diabetes mellitus in Egyptians," *J Genet Eng Biotechnol*, vol. 14, no. 2, pp. 247-251, 2016. Available: https://doi.org/10.1016/j.jgeb.2016.10.004

[13] S. Ereqat, A. Nasereddin, S. Cauchi, K. Azmi, Z. Abdeen, and R. Amin, "Association of a common variant in TCF7L2 gene with type 2 diabetes mellitus in the Palestinian population," *Acta Diabetol*, vol. 47, no. suppl. 1, pp. 195-198, 2010. Available: https://doi.org/10.1007/s00592-009-0161-0

[14] K. Faerch, K. Pilgaard, F. K. Knop, T. Hansen, O. Pedersen, T. Jørgensen, and J. J. Holst, "Incretin and pancreatic hormone secretion in Caucasian non-diabetic carriers of the TCF7L2 rs7903146 risk T allele," *Diabetes Obes Metab*, vol. 15, no. 1, pp. 91-95, 2013. Available: https://doi.org/10.1111/j.1463-1326.2012.01675.x

[15] A. L. Gloyn, M. N. Weedon, K. R. Owen, M. J. Turner, B. A. Knight, G. Hitman et al., "Large-scale association studies of variants in genes encoding the pancreatic β-cell KATP channel subunits Kir6.2 (KCNJ11) and SUR1 (ABCC8) confirm that the KCNJ11 E23K variant is associated with type 2 diabetes," *Diabetes*, vol. 52, no. 2, pp. 568-572, 2003. Available: https://doi.org/10.2337/diabetes.52.2.568

[16] S. F. A. Grant, G. Thorleifsson, I. Reynisdottir, R. Benediktsson, A. Manolescu, J. Sainz J. et al., "Variant of transcription factor 7-like 2 (TCF7L2) gene confers risk of type 2 diabetes," *Nat Genet*, vol. 38, no. 3, pp. 320-323, 2006. Available: https://doi.org/10.1038/ng1732

[17] C. J. Groves, E. Zeggini, J. Minton, T. M. Frayling, M. N. Weedon, N. W. Rayner et al., "Association analysis of 6,736 U.K. subjects provides replication and confirms TCF7L2 as a type 2 diabetes susceptibility gene with a substantial effect on individual risk," *Diabetes*, vol. 55, no. 9, pp. 2640-2644, 2006. Available: https://doi.org/10.2337/db06-0355

[18] R. Gupta, and A. Misra, "Type 2 diabetes in India: Regional disparities," *Br J Diabetes Vasc Dis*, vol. 7, no. 1, pp. 12-16, 2007. Available: https://doi.org/10.1177/14746514070070010301

[19] T. Hayashi, Y. Iwamoto, K. Kaku, H. Hirose, and S. Maeda, "Replication study for the association of TCF7L2 with susceptibility to type 2 diabetes in a Japanese population," *Diabetologia*, vol. 50, no. 5, pp. 980-984, 2007. Available: https://doi.org/10.1007/s00125-007-0618-z

[20] A. Helgason, S. Pálsson, G. Thorleifsson, S. F. A. Grant, V. Emilsson, S. Gunnarsdottir et al., "Refining the impact of TCF7L2 gene variants on type 2 diabetes and adaptive evolution," *Nat Genet,* vol. 39, no. 2, pp. 218-225, 2007. Available: https://doi.org/10.1038/ng1960

[21] S. E. Humphries, D. Gable, J. A. Cooper, H. Ireland, J. W. Stephens, S. J. Hurel et al., "Common variants in the TCF7L2 gene and predisposition to type 2 diabetes in UK European Whites, Indian Asians and Afro-Caribbean men and women," *J Mol Med*, vol. 84, no. 12, pp. 1-10, 2006. Available: https://doi.org/10.1007/s00109-006-0108-7

[22] W. Ip, Y-T. A. Chiang, and T. Jin, "The involvement of the wnt signaling pathway and TCF7L2 in diabetes mellitus: The current understanding, dispute, and perspective," *Cell and Bioscience*, vol. 2, no. 1, pp. 1-12, 2012. Available: https://doi.org/10.1186/2045-3701-2-28

[23] I. A. Khan, P. Jahan, Q. Hasan, and P. Rao, "Genetic confirmation of T2DM meta-analysis variants studied in gestational diabetes mellitus in an Indian population," *Diabetes Metab Syndr*, vol. 13, no. 1, pp. 688-694, 2019. Available: https://doi.org/10.1016/j.dsx.2018.11.035

[24] K. Kirchhoff, F. Machicao, A. Haupt, S. A. Schäfer, O. Tschritter, H. Staiger et al., "Polymorphisms in the TCF7L2, CDKAL1 and SLC30A8 genes are associated with impaired proinsulin conversion," *Diabetologia*, vol. 51, no. 4, pp. 597-601, 2008. Available: https://doi.org/10.1007/s00125-008-0926-y

[25] L. Li., J. Wang, Z. Ping, Y. Li, C. Wang, Y. Shi et al., "Interaction analysis of gene variants of TCF7L2 and body mass index and waist circumference on type 2 diabetes," *Clin Nutr*, vol. 39, no. 1, pp. 192-197, 2019. Available: https://doi.org/10.1016/j.clnu.2019.01.014

[26] V. Lyssenko, R. Lupi, P. Marchetti, S. D. Guerra, M. Orho-Melander, P. Almgren et al., "Mechanisms by which common variants in the TCF7L2 gene increase risk of type 2 diabetes," *J Clin Investig*, vol. 117, no. 8, pp. 2155-2163, 2007. Available: https://doi.org/10.1172/JCI30706

[27] S. A. Miller, D. D. Dykes, and H. F. Polesky, "A simple salting out procedure for extracting DNA from human nucleated cells," *Nucleic Acids Res*, vol. 16, no. 3, pp. 12-15, 1988. Available: https://doi.org/10.1093/nar/16.3.1215

[28] V. Mohan, and R. Deepa, "Adipocytokines and the expanding Asian Indian phenotype," *J Assoc Physicians India*, vol. 54, no. 9, pp. 685-686, 2006.

[29] J. Munoz, K. H. Lok, B. A. Gower, J. R. Fernandez, G. R. Hunter, C. Lara-Castro et al., "Polymorphism in the transcription factor 7-Like 2 (TCF7L2) gene is associated with reduced insulin secretion in nondiabetic women," *Diabetes*, vol. 55, no. 12, pp. 3630-3634, 2006. Available: https://doi.org/10.2337/db06-0574

[30] A. Palizban, M. Nikpour, R. Salehi, and M. R. Maracy, "Association of a common variant in TCF7L2 gene with type 2 diabetes mellitus in a Persian population," *Clin Exp Med*, vol. 12, no. 2, pp. 115-119, 2012. Available: https://doi.org/10.1007/s10238-011-0144-7

[31] S. Peng, Y. Zhu, B. Lü, F. Xu, X. Li, and M. Lai, "TCF7L2 gene polymorphisms and type 2 diabetes risk: A comprehensive and updated meta-analysis involving 121,174 subjects," *Mutagenesis*, vol. 28, no. 1, pp. 25-37, 2013. Available: https://doi.org/10.1093/mutage/ges048

[32] S. D. Rees, S. Bellary, A. C. Britten, J. P. O'Hare, S. Kumar, A. H. Barnett, and M. A. Kelly, "Common variants of the TCF7L2 gene are associated with increased risk of type 2 diabetes mellitus in a UK-resident South Asian population," *BMC Med Genet*, vol. 9, pp. 2-7, 2008. Available: https://doi.org/10.1186/1471-2350-9-8

[33] M. Ridderstråle, and L. Groop, "Genetic dissection of type 2 diabetes," *Mol Cell Endocrinol*, vol. 297, no. 1-2, pp. 10-17, 2009. Available: https://doi.org/10.1016/j.mce.2008.10.002

[34] I. C. Rulifson, S. K. Karnik, P. W. Heiser, D. Ten Berge, H. Chen, X. Gu et al., "Wnt signaling regulates pancreatic β cell proliferation," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 104, no. 15, pp. 6247-6252, 2007. Available: https://doi.org/10.1073/pnas.0701509104

[35] H. Saadi, N. Nagelkerke, S. G. Carruthers, S. Benedict, S. Abdulkhalek, R. Reed et al., "Association of TCF7L2 polymorphism with diabetes mellitus, metabolic syndrome, and markers of beta cell function and insulin resistance in a population-based sample of Emirati subjects," *Diabetes Res Clin Pract*, vol. 80, no. 3, pp. 392-398, 2008. Available: https://doi.org/10.1016/j.diabres.2008.01.008

[36] P. Saeedi, I. Petersohn, P. Salpea, B. Malanda, S. Karuranga, N. Unwin et al. "Global and regional diabetes prevalence estimates for 2019 and projections for 2030 and 2045: Results from the International Diabetes Federation Diabetes Atlas, 9th edition," *Diabetes Res Clin Pract*, vol. 157, 2019, Art. no. 107843. Available: https://doi.org/10.1016/j.diabres.2019.107843

[37] D. K. Sanghera, L. Ortega, S. Han, J. Singh, S. K. Ralhanl, G. S. Wander et al., "Impact of nine common type 2 diabetes risk polymorphisms in Asian Indian Sikhs: PPARG2 (Pro12Ala), IGF2BP2, TCF7L2 and FTO variants confer a significant risk," *BMC Med. Genet*, vol. 9, no. 101, pp. 1-9, 2008. Available: https://doi.org/10.1186/1471-2350-9-59

[38] S. A. Schäfer, O. Tschritter, F. Machicao, C. Thamer, N. Stefan, B. Gallwitz et al. "Impaired glucagon-like peptide-1-induced insulin secretion in carriers of transcription factor 7-like 2 (TCF7L2) gene polymorphisms," *Diabetologia*, vol. 50, no. 12, pp. 2443-2450, 2007. Available: https://doi.org/10.1007/s00125-007-0753-6

[39] R. Sladek, G. Rocheleau, J. Rung, C. Dina, J. Shen, D. Serre et al. "A genome-wide association study identifies novel risk loci for type 2 diabetes," *Nature*, vol. 445, no. 7130, pp. 881-885, 2007. Available: https://doi.org/10.1038/nature05616

[40] F. J. Tsai, C. F. Yang, C. C. Chen, L. M. Chuang, C. H. Lu, C. T. Chang et al. "A genome-wide association study identifies susceptibility variants for type 2 diabetes in Han Chinese," *PLoS Genet*, vol. 6, no. 2, 2010. Available: https://doi.org/10.1371/journal.pgen.1000847

[41] A. Turki, G. S. Al-Zaben, N. Mtiraoui, H. Marmmuoch, T. Mahjoub, and W. Y. Almawi, "Transcription factor-7-like 2 gene variants are strongly associated with type 2 diabetes in Tunisian Arab subjects," *Gene*, vol. 513, no. 2, pp. 244-248, 2013. Available: https://doi.org/10.1016/j.gene.2012.10.086

[42] L. Ugozzoli, and R. B. Wallace, "Allele-specific polymerase chain reaction," *Methods,* vol. 2, no. 1,

pp. 42-48, 1991. Available: https://doi.org/10.1016/S1046-2023(05)80124-0

[43] R. Q. Wang, D. H. Zhou, B. Xi, X. S. Ge, P. Zhu, B. Wang et al. "ENPP1/PC-1 gene K121Q polymorphism is associated with obesity in european adult populations: Evidence from a meta-analysis involving 24,324 subjects," *Biomed Environ. Sci*, vol. 24, no. 2, pp. 200-206, 2011. Available: https://doi.org/10.3967/0895-3988.2011.02.015

[44] K. V. Yazdi, S. M. Kalantar, M. Houshmand, M. Rahmanian, M. R. Manaviat, M. R. Jahani et al., "SLC30A8, CDKAL1, TCF7L2, KCNQ1 and IGF2BP2 are associated with type 2 diabetes mellitus in Iranian patients," *Diabetes Metab Syndr Obes,* vol. 13, pp. 897-906, 2020. Available: https://doi.org/10.2147/DMSO.S225968

[45] E. Zeggini, M. N. Weedon, C. M. Lindgren, T. M. Frayling, K. S. Elliott, H. Lango et al., "Replication of genome-wide association signals in UK samples reveals risk loci for type 2 diabetes," *Science*, vol. 316, no. 5829, pp. 1336-1341, 2007. Available: https://doi.org/10.1126/science.1142364