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Research Article

GENETIC SUSCEPTIBILITY OF TRANSCRIPTION FACTOR 7-LIKE 2 GENE VARIANT AND RISK OF TYPE 2 DIABETES IN ASIAN INDIANS

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ABSTRACT

Objectives: The variants of transcription factor 7-like 2 (*TCF7L2*) gene have been shown to be associated with type 2 diabetes mellitus (T2DM) and its related complications.
We aimed to explore the possible association of rs7903146 (C/T) variant in *TCF7L2* with the risk of T2DM in the North Indian population.

Methods: The present case–control study included a total of 638 human subjects (318 T2DM subjects and 320 healthy controls). Various anthropometric, biochemical, and genetic parameters were studies in all the subjects. Genotyping of *TCF7L2* gene was carried out using allele-specific polymerase chain reaction method.

Results: The results of this study indicate significantly higher values of body mass index, waist circumference, waist-to-hip ratio, and body fat (%) in T2DM subjects than controls (p≤0.001). Dyslipidemia represented by higher levels of triglycerides and reduced values of high-density lipoprotein was more predominant in diabetic subjects compared to healthy subjects. The frequency of risk genotype (TT) frequency was significantly higher in T2DM subjects (16.4%) compared to controls (11.6%). The "T" allele was more dominant in diabetic subjects than controls. Logistic regression analysis of the data revealed a significant association of TT genotype with 2-fold (odds ratio with 95% of confidence interval; 2.09 [1.29–3.42] p=0.003) and CT genotype with 1.7-fold (1.73 [1.23–2.44] p=0.002) increased risk of developing T2DM.

Conclusions: The present study demonstrated a significant association of rs7903146 (C/T) variant in *TCF7L2* with the augmented risk of T2DM in North Indian population.

Keywords: Single-nucleotide polymorphism, Transcription factor 7-like 2, Type 2 diabetes mellitus, Insulin resistance, Asian Indians.

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INTRODUCTION

Type 2 diabetes mellitus (T2DM) is one of the most challenging problems of the 20th century. T2DM is characterized by hyperglycemia, impaired insulin secretion or insulin resistance, is the most common type of diabetes accounting 90–95% of total diabetic cases. The prevalence of T2DM is rising rapidly across the globe posing significant socioeconomic burden in the form of lost productivity and stress on health-care system, both in developed and developing countries [1]. At present, T2DM affects 285 million people all over the world and is predicted to rise to 642 million by 2040 globally [2]. At present, India has 85 million individuals suffering from T2DM; however, this number is expected to rise to 109 million by 2035 [3]. India has become diabetic capital of the world. About 80% of the diabetic population lives in low- and middle-income countries. T2DM is a very complex metabolic disease in which both genetic and environmental factors play an important role in their pathophysiology [4]. The recent advancement in technology had made it possible to identify new genetic loci as well as genes associated with the risk of the development of T2DM. These genetic determinants can be used to better understanding of the pathogenesis of a disease, help in the development of policies to counter the economic burden, and provide a new way for improved and preventive therapeutic measures.

Transcription factor 7-like 2 (*TCF7L2*) gene is located on the long arm of chromosome 10q25.3 and involved in Wnt signaling pathway which plays a pivotal role in cell development and growth regulation [5]. The previous studies established *TCF7L2* gene as possible determinants of type 2 diabetes [6,7]. Although several single-nucleotide polymorphisms (SNPs) in *TCF7L2* gene have been replicated in different population and ethnicities, few studies have been done in Indian population. Furthermore, genetic association of *TCF7L2* gene has not yet been explored in North Indian population. Hence, the present study was planned to investigate the association of rs7903146 (C/T) variant in *TCF7L2* with the risk of T2DM in North Indian population.

METHODS

Study population

The present study included 638 participants (318 T2DM patients and 320 healthy controls) recruited from North Indian population. The diagnosis of T2DM was done using criteria established by the American Diabetes Association as follows: A medical record indicating either a fasting glucose levels >7.0 mmol/l or >126 mg/dl after a minimum 12-h fast or 2-h post-glucose level (oral glucose tolerance test or 2-h) >11.1 mmol/l or >200 mg/dl on more than one occasion with symptoms of diabetes. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. This study was ethically approved by the Institutional Ethics Committees of Post Graduate Institute of Medical Education

and Research, Chandigarh and Panjab University, Chandigarh, India. A model consent form adhering to Indian, and International guidelines regarding the use of human subjects were used along with detailed questionnaire of details regarding demographic and socioeconomic characteristics. The participant's self-reported age, sex, educational status, physical activity, dietary habits, family history of the disease and individual's smoking and alcohol use, etc., were recorded.

Anthropometric measurements

Standard anthropometric measurements were performed including stature, weight, and waist and hip circumferences. Body mass index (BMI) was calculated according to Quetelet equation (BMI = weight in kilograms/height in meters squared). Waist-to-hip ratio (WHR) was calculated as ratio of abdomen to hip circumferences. Blood pressure (BP) was measured using Omron's BP machine in a sitting position, from the left arm resting on the table, with legs uncrossed and feet flat.

The abdominal obesity was measured according to the new cutoffs proposed for South Asian Indians as mentioned in our previous study [8], i.e., WHR >0.89 for men and >0.81 for women. BMI <23 kg/m^2 has been proposed for low risk, 23–27.5 kg/m^2 for increased risk, and ≥27.5 kg/m^2 for high risk for developing weight-related diseases in Asian populations.

Clinical parameters

Venous blood samples were extracted from each subject after 12 h of fasting. A serum sample was analyzed for fasting serum glucose, creatinine, and lipid profile (triglycerides [TG], total cholesterol [TC], high-density lipoprotein cholesterol [HDL-C], and low-density lipoprotein cholesterol [LDL-C]). Fasting and random blood glucose levels were measured using a portable glucometer (Abbott Optium Xceed, USA).

Genotyping of *TCF7L2* gene

The genomic DNA was isolated from the blood using salting out method [9]. DNA yield was measured by absorbance at 260 nm and purity was checked by calculating A_{260}/A_{280}. Amplification of rs7903146 polymorphism in *TCF7L2* gene was carried out using allele-specific polymerase chain reaction (PCR) as described in an earlier study [10]. Genotyping was based on differential amplification due to the presence of mismatches. In rs7903146 genotyping, two forward primers with a mismatch in their last 3' nucleotide such a way that each is specific for one of the two variants of the polymorphism and a common reverse primer was used. The forward primers also contain a second mismatch at the third nucleotide from the 3′ end to enhance PCR specificity. The forward primers (rs7903146 C or rs7903146 T) specific for allele C detection: 5`GAACAATTAGAGA GCTAAGCACTTTTTAGAAAC 3` and forward primer for allele T detection: 5`GAACAATTAGAGAGCTAAGCACTTTTTAGAGAT 3`, and common reverse primer (rs7903146 R) 5` AGATGAAATGTAGCAGTGAAGTGC 3` were combined in two parallel PCR reactions one with primers rs7903146 C and rs7903146 R (PCR C) and a second with primers rs7903146 T and rs7903146 R (PCR T). For each sample, two PCR reactions were run in parallel, one with primers rs7903146 C and rs7903146 R (PCR C) and a second with primers rs7903146 T and rs7903146 R (PCR T) each containing 200 ng genomic DNA, 1X Taq polymerase buffer, 1.5 mM $MgCl_2$, 10 pmol of each primer, 200 µmol/l dNTPs, and 1U of Taq DNA polymerase (Thermo) in a final volume of 25 µl. DNA amplification was carried out on thermal cycler (Eppendorf Mastercycler Nexus Gradient) with an initial denaturation for 3 min at 94°C, followed by 32 cycles of 1 min at 94°C, 1 min at 50°C, and 1 min at 72°C and final extension for 5 min at 72°C. Fig. 1 shows the amplified PCR products separated on 1.5% agarose gel electrophoresis.

Statistical analysis

Results were expressed as mean ± standard deviation. Chi-square analysis was applied to test the significance of differences in genotypic and allelic frequencies. Group comparisons were done using unpaired t-tests. p<0.05 (two tailed) was considered as statistically significant difference. Logistic regression analyses were performed to calculate odds ratio (OR) and 95% confidence intervals (CIs) for each risk factor. Statistical analysis was performed using IBM-SPSS for Windows, version 20 (SPSS, Inc., Chicago, IL).

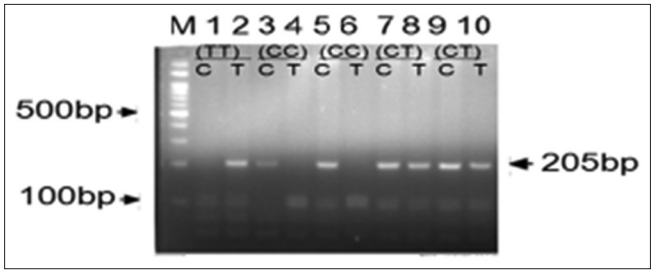

Fig. 1: Agarose gel electrophoresis of transcription factor 7-like 2 (rs7903146C/T) gene polymorphism. Lane M: 100 bp marker ladder; lanes (1,2) (5,6): CG genotype; lanes (3,4): GC genotype; lanes (7,8) (9,10): GG genotype

RESULTS

Clinical and biochemical characteristics of the study subjects

Table 1 shows the clinical and biochemical characteristics of the study subjects. There was a no significant difference in the age of the diabetic and control subjects (47.13±11.7 vs. 55.3±11.3, p=0.001). T2DM subjects show predominant abdominal obesity reflected by significantly higher values of BMI (26.9±4.6 vs. 25.9±4.7, p=0.02), waist circumference (93.1±10.5 vs. 88.1±10.8, p=0.001), and waist to hip circumference (0.96±0.06 vs. 0.93±0.08, p=0.001). No significant difference in body fat percent was observed in T2DM subjects compared to controls (33.7±10.3 vs. 32.7±9.1). Although TC levels fall under the limit of borderline, there is no significant difference between diabetic and non-diabetic controls. Significantly higher values of TG and reduced values of HDL were reported in diabetic patients than controls. Along with abdominal obesity evidenced by higher BMI and WHR, 12% of patients were having dyslipidemia and were on lipid-lowering drugs. Alterations in clinical and anthropometric measurements pretend a risk for the development of cardiac diseases as demonstrated by higher TC/HDL, LDL/HDL, and TG/HDL ratio in diabetic subjects compared to healthy controls (Table 1).

Association of *TCF7L2* gene polymorphisms with T2DM

The distribution of genotype and allelic frequencies for rs7903146 polymorphisms of *TCF7L2* gene are shown in Table 2. *TCF7L2* rs7903146 gene polymorphism analysis demonstrated that the frequency of risk genotype TT genotype was significantly higher in diabetics than in controls (16.4% vs. 11.6%). The frequency of the "T" allele was significantly higher in diabetic subjects (42%) compared with that in the healthy control subjects (33%). Furthermore, CT genotype frequency was more predominant in T2DM subjects (51.3%) than control subjects (42.2%). Logistic regression analysis of the data demonstrated a significant association of TT genotype with 2-fold (OR with 95% of CI; 2.09 [1.29–3.42] p=0.003) and CT genotype with 1.7 fold (1.73 [1.23–2.44] p=0.002) increased risk of developing T2DM in this population. Under a dominant model of inheritance, T allele shows a significant association with type 2 diabetes (OR, 1.79 [1.30–2.47] p=0.0004). Furthermore, no significant association was observed with T2DM under recessive model of inheritance (1.49 [0.95–2.35] p=0.08).

Table 3 summarizes the comparison of clinical and biochemical characteristics of T2DM and control subjects according to different genotypes of *TCF7L2* rs7903146. There were no significant differences in metabolic characteristics such as glucose, BMI, WC, WHR, body fat (%), systolic BP, diastolic BP, TC, TG, HDL, LDL, very LDL, and creatinine among T2DM as well as control subjects carrying CC, CT, and TT genotypes of rs7903146 polymorphisms in *TCF7L2* gene.

Table 1: Comparison of anthropometric and clinical characteristics of the study subjects

Parameters	Controls	T2DM patients	p-value
	Mean±SD	Mean±SD	
BMI (kg/m²)	25.99±4.7	26.87±4.6	0.02*
Waist circumference (cm)	88.14±10.8	93.13±10.5	0.00*
Hip (cm)	95.18±10.1	96.91±10.3	0.03*
WHR	0.93±0.08	0.96±0.06	0.00*
Body fat (%)	32.69±9.1	33.71±10.3	0.18
SBP (mmHg)	115.97±8.2	130.78±15.3	0.00*
DBP (mmHg)	75.94±6.6	79.81±10.4	0.00*
Glucose (mg/dl)	92.26±11.0	146.49±53.2	0.00*
TC (mg/dl)	177.69±31.6	183.33±48.7	0.10
TG (mg/dl)	149.50±55.2	172.49±87.7	0.00*
HDL-C (mg/dl)	44.77±6.2	42.21±7.3	0.00*
LDL-C (mg/dl)	103.02±28.3	106.62±42.8	0.23
VLDL-C (mg/dl)	29.90±11.0	34.50±17.5	0.00*
Creatinine (mg/dl)	0.81±0.6	0.88±0.4	0.14
Total lipids (mg/dl)	504.87±102.7	539.15±158.8	0.00*
Castelli's risk index I (TC/HDL)	4.05±0.9	4.47±1.4	0.00*
Castelli's risk index II (LDL/HDL)	2.36±0.8	2.61±1.2	0.00*
Atherogenic coefficient (TG/HDL)	3.42±1.4	4.27±2.4	0.00*
Atherogenic index	0.50±0.2	0.57±0.2	0.00*

Data values are represented as mean±SD. BMI: Body mass index, SBP: Systolic blood pressure, DBP: Diastolic blood pressure, TC: Total cholesterol, HDL-C: High-density lipoprotein cholesterol, LDL: Low-density lipoprotein, VLDL: Very low-density lipoprotein, WHR: Waist-to-hip ratio, T2DM: Type 2 diabetes mellitus, TG: Triglyceride, SD: Standard deviation. *Significant difference between T2DM and control subjects. p<0.05 is considered as statistically significant value

Table 2: Test of association depicting *TCF7L2* (rs7903146) gene polymorphism as a risk for T2DM in North Indian population

Genotypes	Human participants		Test of association	
	Controls	T2DM patients	Odds ratio (95% of CI)	Pearson Chi-square
CC	148 (46.2%)	103 (32.4%)	Reference	
CT	135 (42.2%)	163 (51.3%)	1.73 (1.23–2.44) p=0.002	10.18
TT	37 (11.6%)	52 (16.4%)	2.09 (1.29–3.42) p=0.003	9.01
Total	320	318		
Allele C	0.67	0.58		
Allele T	0.33	0.42		
Dominant model (CC vs. CT+TT)				
CC	148	103		
CT+TT	172	215	1.79 (1.30–2.47) p=0.0004	12.84
Recessive model (CC+CT vs. TT)				
CC+CT	283	266		
TT	37	52	1.49 (0.95–2.35) p=0.08	3.05

Data are presented as number (%) unless otherwise stated. T2DM: Type 2 diabetes mellitus, CI: Confidence interval, *TCF7L2*: Transcription factor 7-like 2. p<0.05 is considered as statistically significant value

DISCUSSION

India is currently experiencing an epidemic of DM. Both environmental and genetic factors contribute to the development of insulin resistance and type 2 diabetes [11,12]. It is evident from the previous studies that several genetic determinants are associated with increased risk of type 2 diabetes, but their conclusive role is still unclear [13]. *TCF7L2* gene is considered one of the most important candidate genes for T2DM, playing a key role in blood glucose homeostasis and beta-cell function [14]. *TCFL2* encodes a basic helix-loop-helix TCF-4, which acts as a nuclear receptor for the Wnt/β-catenin pathway [15] and can preferentially bind to Wnt-responsive elements in genes induced by β-catenin [16]. It is well known that the β-catenin/TCF-4 complex participates in various biological events. Particularly, the complex has been found to have an important role in pancreas islet cell proliferation and differentiation and thus contributes to T2DM initiation and progression.

Following the initial report by Grant *et al.* [17] showing that *TCF7L2* variants were strongly associated with T2DM risk, several other studies consistently replicated this association in different ethnicities [6]. The present case–control study established the association of 2-fold increased diabetes risk with TT homozygous, while CT heterozygous carried 1.7-fold increase in T2DM risk when compared with CC homozygous of rs7903146 polymorphisms in the *TCF7L2* gene. Bodhini *et al.* observed a significant association between the T allele of rs7903146(C/T) SNPs and T2DM in South Indians [18]. Chandak *et al.* also observed a strong association of rs7903146 polymorphism with T2DM (OR=1.46) [19]. A previous study revealed a positive significant association between TT genotype of rs7903146 (C/T) variant of *TCF7L2* gene and diabetes-related complications in Indian population [20]. Several studies conducted in other parts of the world demonstrated significant relationship between *TCF7L2* gene and T2DM in British [21], the US [22], Finnish [23], Amish [24], Scandinavian [25], Polish [25], French [26], Dutch Breda [27], European Whites, migrant Asian Indian, Afro-Caribbean [28], Northern Swedish [29], and Japanese populations [30]. A large meta-analysis study confirmed the association of *TCF7L2* gene with T2DM in different ethnicities [31]. Our results are similar with the results from the overall meta-analysis of the rs7903146 polymorphism wherein heterozygous genotype CT carried over a 1.4-fold increased risk for T2DM, while TT homozygous carried near a 2.0-fold increase in T2DM risk when compared with CC homozygous. Another meta-analysis study examining 66 studies also confirmed the association of the rs7903146 SNPs with T2DM in 66 studies (OR = 1.41, 95% CI 1.37–1.46 for the T allele) [32]. A similar meta-analysis study in Chinese population also reflected the role of rs7903146(C/T) SNP towards

Table 3: Metabolic characteristics stratified according to the genotypes of *TCF7L2* rs7903146 polymorphism in controls and T2DM subjects

Parameters	Control subjects				T2DM subjects			
	CC (n=148)	CT (n=135)	TT (n=37)	p value	CC (n=103)	CT (n=163)	TT (n=54)	p-value
	Mean±SD	Mean±SD	Mean±SD		Mean±SD	Mean±SD	Mean±SD	
BMI (kg/m$^{2)}$	25.61±4.8	26.37±4.6	26.17±4.4	0.381	26.46±4.8	27.19±4.5	26.71±4.2	0.428
WC (cm)	88.96±11.3	87.58±10.3	86.94±10.8	0.437	91.72±9.8	93.64±11.5	94.33±8.5	0.235
Hip (cm)	94.74±10.0	95.78±10.5	94.71±9.4	0.664	95.47±9.4	97.82±11.2	96.93±8.8	0.193
WHR	0.94±0.08	0.92±0.1	0.92±0.07	0.033	0.96±0.1	0.96±0.1	0.97±0.1	0.245
Body fat (%)	32.09±9.4	33.79±8.4	31.05±10.0	0.145	31.57±10.0	35.23±10.6	33.19±9.3	0.017
SBP (mmHg)	116.22±8.1	115.68±6.9	116.03±12.1	0.859	130.85±15.2	130.07±15.9	132.85±13.7	0.524
DBP (mmHg)	75.53±6.7	76.59±6.7	75.17±6.1	0.313	80.91±13.7	79.26±8.7	79.33±7.4	0.424
Glucose (mg/dl)	91.98±10.4	93.07±11.2	90.46±12.4	0.427	151.56±58.1	142.31±51.5	149.76±47.7	0.349
TC (mg/dl)	177.99±31.5	177.51±31.4	177.12±33.6	0.986	177.73±47.1	185.59±51.4	187.46±42.4	0.387
TG (mg/dl)	154.87±62.6	146.02±48.0	140.56±46.5	0.26	171.86±81.8	175.57±97.2	163.38±63.1	0.713
HDL-C (mg/dl)	44.51±6.1	44.68±5.5	46.13±9.0	0.391	42.23±6.6	42.41±7.5	41.50±8.0	0.765
LDL-C (mg/dl)	102.51±28.1	103.62±27.7	102.87±32.0	0.95	101.13±42.9	108.07±44.3	113.28±36.9	0.244
VLDL-C (mg/dl)	30.97±12.5	29.20±9.6	28.11±9.3	0.26	34.37±16.4	35.11±19.4	32.68±12.6	0.713
Atherogenic index	0.52±0.2	0.50±0.2	0.47±0.2	0.262	0.57±0.2	0.57±0.2	0.57±0.2	0.971
Creatinine (mg/dl)	0.85±0.9	0.77±0.3	0.81±0.3	0.608	0.86±0.4	0.91±0.5	0.81±0.5	0.373

Data values are written as mean±SD, SBP: Systolic blood pressure, DBP: Diastolic blood pressure, TC: Total cholesterol, TG: Triglyceride, WC: Waist circumference, WHR: Waist-to-hip ratio, BMI: Body mass index, HDL: High-density lipoprotein, LDL: Low-density lipoprotein, VLDL: Very low-density lipoprotein, *TCF7L2*: Transcription factor 7-like 2, SD: Standard deviation

increased susceptibility to T2DM (OR = 1.54, 95% CI 1.37- 1.74 for the T allele) [33]. A study by Lou *et al.* indicated that four SNPs of *TCF7L2* (rs7903146, rs12255372, rs11196205, and rs290487) were associated with T2DM risk in East Asian [34]. Eight *TCF7L2* polymorphisms in 155 studies with 121,174 subjects (53,385 cases and 67,789 controls) were addressed in their meta-analysis. Significant association was established between T2DM risk and rs7903146 and many other polymorphisms in *TCF7L2* gene under an additive inheritance model.

The present study did not find any link between SNP rs7903146 and abnormalities in cardiometabolic traits including BMI, WHR, and dyslipidemia. Similar results were observed in the previous studies conducted in India. Chandak *et al.* observed no significant association with BMI or WHR in T2DM patients or control subjects [19]. Also, no significant difference in respect to metabolic parameters between different genotypes of rs7903146(C/T was observed [36]. Contrary, many studies show the association of *TCF7L2* gene polymorphism with many metabolic traits [6,17,35-38]. Cauchi *et al.* found that the T allele of rs7903146 predicts hyperglycemia in French population [26]. Saxena *et al.* found that individuals homozygous for the rs7903146 risk allele had a significant reduction in insulinogenic index and insulin disposition index [25]. Damcott *et al.* found significant association with insulin sensitivity index, acute insulin response to glucose in non-Amish Caucasian subjects [24]. Our results showed that the rs7903146 T allele of the *TCF7L2* gene was positively correlated with an enhanced risk of T2DM in codominant and dominant models of inheritance.

CONCLUSIONS

The present study confirms the association of rs7903146 (C/T) polymorphism in *TCF7L2* gene with risk of T2DM in North Indian population. However, no significant influence of this polymorphism was observed with cardiometabolic traits.

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AUTHORS' CONTRIBUTIONS

Study design and implementation: GKB, SS, SKB, and JSB. Data collection and analysis: GKB and SKB. Manuscript drafting: NK, GKB, and JSB. Manuscript revisions: All authors.

CONFLICTS OF INTEREST

The authors have no conflicts of interest to declare.

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