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Influence of *KCNQ1* and *TCF7L2* genes associated with the role of type 2 diabetes

Nada Alqadri [1], Nuha A. Abdelmutalab [2], Sitalnesa Abdelhafeez [3], Atyah Y. Alzahrani [4], Omima Gadalla Mohamed [3], Aeshah Hassan [5], Omaima Nasir [1,*]

[1]*Department of Biology, Turabah University College, Taif University, P.O. Box 11099, Taif 21944, Saudi Arabia*
[2]*Department of Infection Control, King Faisal Medical Complex. P.O. Box 2265, Taif 21944, Saudi Arabia*
[3]*Department of Communicable Diseases Control Directorate, Taif 21944, Saudi Arabia*
[4]*Department of Family Medicine, Directorate, Taif 21944, Saudi Arabia*
[5]*Department of Clinical Laboratory Sciences, Turabah University College, Taif University, P.O. Box 11099, Taif 21944, Saudi Arabia*

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ABSTRACT

T2DM is a long-term metabolic disorder characterized by either the pancreas' inability to produce enough insulin or the body's inability to properly utilize the insulin it produces. The β-cell function and blood glucose homeostasis are two areas where *TCF7L2* (Transcription factor 7 like 2) appears to be a significant candidate gene. *KCNQ1* (potassium voltage-gated channel subfamily, member 1 has been discovered as a T2DM susceptibility gene in Asian populations by genome-wide association studies with rs2237892 polymorphism and an increased risk of developing T2DM. The aim of this study was to investigate the association between rs7903146 and rs2237892 SNP studies in T2DM patients. In this study, 60 T2DM cases and 60 controls were selected. Genotyping was performed for rs7903146 and rs2237892 SNPs using specific primers and restriction enzymes, then all PCR products were loaded on an agarose gel stained with ethidium bromide. The current study results confirmed rs7903146 SNP was strongly associated with genotype (OR-4.14; 95%CI:1.07-15.98; p=0.02) and allele frequencies (OR-4.60; 95%CI:1.66-12.70; p=0.001) whereas in rs2237892 SNP was not associated with any of the genotypes (OR-4.29; 95%CI:0.46-39.58; p=0.16; OR-3.21) or allele frequencies (OR-6.26; 95%CI:0.74-52.83; p=0.055). The current study results were found to be associated with global studies carried out in rs7903146 and rs2237892 SNP. The strength of this current study was to involve Saudi nationalities and we have screened rs7903146 and rs2237892 SNPs which plays a major role in T2DM. Involving 60 T2DM cases/60 controls was the major limitation of this study. Missing validation through Sanger sequencing analysis was one of the limitations of this study. In conclusion, the current study results confirmed rs7903146 SNP was strongly associated with T2DM and rs2237892 SNP was not associated with T2DM patients.

1. Introduction

Diabetes Mellitus is a set of metabolic disorders characterized by increased serum glucose levels or persistent hyperglycemia, resulting in abnormalities of insulin production, insulin action, or both. Type 1 diabetes is defined by the production of little or no insulin, which further affects immune system cells. Pre-diabetes is defined as having blood sugar levels that are higher than usual but not high enough to warrant a diagnosis of permanent diabetes (Khan, 2021). Type 2 diabetes mellitus (T2DM) is a developing global epidemic that, until recently, was thought to be chronic and progressing in nature. Despite the fact that lifestyle and dietary changes are at the core of treatment, pharmacological medications have been employed to optimize glycemic control (Brown et al., 2022). T2DM is found in 90% of the human population (Khan et al., 2015a).

T2DM is typically diagnosed as a silent prevalent chronic disease that accounts for about 90% of all

* Corresponding Author.
Email Address: o.saeeed@tu.edu.sa (O. Nasir)
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Corresponding author's ORCID profile:
https://orcid.org/0000-0002-3021-6473

diabetic cases (Taheri et al., 2022). Elevated glucose levels are caused by a lack of insulin secretion/resistance (Wang et al., 2022a). T2DM is a risk factor for obesity, gestational diabetes (Alharbi et al., 2022) and renal disease (Jiang et al., 2022). T2DM, or adult-onset diabetes, affects 382 million people worldwide, and that number is expected to rise to 592 million by 2035 when it would affect 130 nations (Alharbi et al., 2021). An estimated 463 million adults aged 20-79 have diabetes, and this number is anticipated to rise to 578.4 million by 2030 and 702.2 million by 2045, according to data published in 2019 by the International Diabetes Federation (Wang et al., 2022b). In addition, the incidence in Saudi Arabia has gone from 7% in 1989 to 32% in 2009, a dramatic increase (Alzaheb and Altemani, 2018). Because of the region's rapid economic development, urbanization, and lifestyle changes, the Middle East and North Africa are predicted to have the greatest overall prevalence of diabetes. Diabetes has become the most important health concern in the Kingdom of Saudi Arabia as a result of the global epidemic.

Diabetes was diagnosed in roughly 0.9 million people in Saudi Arabia in 1992, but by 2010, the number had climbed to 2.5 million, a 27-fold increase in incidence rates in just two decades. In 2015, 4660 patients with diabetes visited family and medical clinics in Saudi Arabia. Diabetes prevalence rises when obesity rates rise and the population ages (Alotaibi et al., 2017). There are two categories of risk factors for T2DM: modifiable and non-modifiable. Consumption of saturated fat and simple carbohydrates, poor glucose tolerance, metabolic syndrome, hypertension, increased plasma triglycerides, and a lack of physical activity are all modifiable risk factors. Age, family history of diabetes, ethnicity, and previous diabetic pregnancy (GDM) are all non-modified risk factors. When T2DM is not adequately treated, it can result in major complications such as nephropathy, neuropathy, and retinopathy, as well as coronary artery disease, peripheral artery disease, and cerebrovascular disease, all of which can be fatal (Fareed et al., 2017).

The genes/single nucleotide polymorphisms (SNPs) associated with T2DM have been extensively studied in genome-wide association studies (GWAS). Transcription factor 7 Like 2 (*TCF7L2*) and potassium voltage-gated channel subfamily Q member 1 (*KCNQ1*) genes are implicated in Wnt signaling pathways and potassium channels associated with diabetes, notably T2DM. *TCF7L2* is a transcription factor involved in the Wnt signaling pathway. *TCF7L2* is active in glucose homeostasis by regulating the expression of the pro-glucagon gene, which encodes glucagon-like peptide 1 in intestinal cells. The *KCNQ1* is connected with K_{ATP} channels, K_v channels, and voltage-dependent Ca^{+2} channels all work together to control pancreatic-cell insulin secretion. Voltage-gated potassium (K_+) channels, also known as $K_v7.1$, are found in many tissues, including the cardiovascular and pancreatic systems, as well as the kidneys (Khan et al., 2015a; 2015b). The rs7903146 and rs2237892 polymorphisms in the *TCF7L2* and *KCNQ1* genes have been systematically explored in the global population of T2DM (Rattanatham et al., 2021) and GDM patients (Ao et al., 2015). The aim of this study was to investigate the role of rs7903146 and rs2237892 polymorphisms in *TCF7L2* and *KCNQ1* genes in T2DM patients of Saudi Arabia.

2. Materials and methods

2.1. Sample Size

The sample size was calculated using the web-based tool† of the Power Calculator for Genetic Studies. We also adopted a multiplicative sickness model and predicted disease allele frequencies of 0.25. This study needed to have at least 85% power to reject the null hypothesis with an odds ratio (OR) of 1.5 and 200 cases and 150 controls (Chehadeh et al., 2016; Alqadri, 2022). However, due to criteria selection in our study, we were only able to include 60 people with T2DM and 60 healthy controls.

2.2. T2DM subjects

A total of 350 participants were recruited in this study and finally, we have opted for 60 T2DM patients and 60 control subjects. Each gender was assigned an equal number of T2DM cases and controls. Between the ages of 50-85, those in the study who had T2DM were included. The 120 participants of both T2DM Cases and healthy controls were collected from various parts of the Countries. The inclusion and exclusion criteria of T2DM cases and controls were defined in our previous study (Alqadri, 2022). Normal glucose levels were used to select healthy controls (n=60), who had no history of diabetes are considered as inclusion criteria. Patients were excluded if they tested positive for any of the following: high blood glucose, other metabolic diseases, or were on metformin. A total of 60 patients with T2DM were selected using American Diabetes Association criteria. Patients with fasting blood glucose (FBG) values more than or equal to 7.0 mmol/l met the criteria for inclusion as T2DM. In order to rule out T2DM, FBG levels should have the low or under the range of impaired fasting glucose or even normal glucose levels were excluded from this study. After or before an average of five years, all diabetic patients in this study were diagnosed with T2DM.

2.3. Sample collection

A heparinized vacutainer tube was used to collect five milliliters of each participant's peripheral blood by venipuncture. A total of 5ml of blood was split for serum analysis (3ml) and DNA isolation (2ml) (Alshammary and Khan, 2021). In 120 patients, anthropometric measurements such as age, gender,

† http://www.sph.umich.edu/csg/abecasis/CaTS/index.html

and body mass index (BMI) were recorded. Hypertension (HTN) was defined as systolic blood pressure (SBP) of more than 140 mmHg and/or diastolic blood pressure (DBP) higher than 90 mmHg (Alharbi et al., 2013)

2.4. Biochemical analysis

Serum was isolated from clotted blood collected in the vacutainer utilized for fasting blood glucose (FBG) and lipid profile assessments. The four parameters in the lipid profile are high-density lipoprotein cholesterol (HDLC), low-density lipoprotein cholesterol (LDLC), triglycerides (TG), and total cholesterol (TC). Blood glucose levels were determined using the serum from plain tubes that had been collected and sent to a clinical chemistry laboratory for analysis. Glycated hemoglobin (Hb1Ac) levels were determined using an EDTA sample obtained from 120 subjects (Alqadri, 2022).

2.5. Molecular investigation

DNA was extracted from 120 EDTA blood vacutainers using commercially available genomic DNA purification kits. Analyzing DNA concentrations and purity necessitated employing the Nanodrop2000 spectrophotometer (Thermo Fisher Scientific Inc., Massachusetts, USA). The isolated DNA was maintained at -20°C for further research (Al-Otaiby et al., 2021). Site-specific restriction enzymes were used to analyze PCR results for polymorphisms in single nucleotide sequences (SNPs) and restriction fragment length polymorphism (RFLPs). Both *TCF7L2* (rs7903146) and *KCNQ1* (rs2237892) gene polymorphisms were amplified using specific oligonucleotide sequence primers (Rattanatham et al., 2021). *TCF7L2* (rs7903146) and *KCNQ1* (rs2237892) gene polymorphisms were subjected to the following PCR protocol: 10 minutes of initial denaturation at 95^0C, 40 cycles of the total reaction involving denaturation at 94^0C for 1 minute, 1 minute of annealing temperature at 62^0C, 1 minute of extension at 72^0C, and a final 10 minutes of 72^0C extension time (Alqadri, 2022). Prior to digestion, the *KCNQ1* (rs2237892) and *TCF7L2* (rs7903146) gene polymorphisms PCR products were amplified with 2µl of *HpaII* and *RsaI* restriction enzymes, respectively, from BioLabs in New England. Then the digested products were resolved on 2.0 percent agarose gels and visualized by UV illumination after staining with Ethidium bromide for fragments of a certain size. For genotype and allele frequency, the size of the bands indicated their interpretation.

2.6. Statistical analysis

The statistical analysis was carried out using SPSS software (25th version, USA). For the student t-test, Hardy Weinberg Equilibrium (HWE), and genotyping analysis for the rs7903146 and rs2237892 polymorphisms between diabetic patients and controls, all clinical and genotype data were collected in Excel and then converted to SPSS files. FBG was used as a reference for multiple nominal regression analysis in diabetic cases. The mean and standard deviation are used to describe continuous variables, while percentages are used to represent categorical variables. A substantial correlation between the two groups is shown by a p-value of less than 0.05 when comparing the two groups (Khan et al., 2019).

3. Results

Table 1 defines demographic information for both control participants and T2DM patients. In this study, 60 T2DM cases and 60 healthy controls were enrolled. Thirty males and thirty females were distributed equally between the two groups. The mean ages of the patients and controls were 63.71±9.81 and 58.1±8.37 years, respectively. When compared to healthy controls, the mean values of weight, BMI, FBG, Hb1Ac, SBP, DBP, LDLC, and TC were higher in T2DM cases ($p<0.05$). Gender, height, HDLC, and TG, on the other hand, were not associated with the groups ($p>0.05$).

Table 1: Clinical and demographical characteristics between control subjects and T2DM cases

	Controls (n=102)	Cases (n=60)	P-value
Age (Years)	58.1±8.37	63.73±9.81	0.009
Gender (F:M)	30:30	30:30	0.00
Height (cms)	156.70±6.87	158.99±7.83	0.09
Weight (kg)	70.1±10.76	78.48±11.88	0.001
BMI (kg/m^2)	28.40±3.46	31.14±4.48	0.0002
FBG (mmol/l)	5.1±1.45	10.69±2.08	<0.0001
Hb1Ac	5.3±0.68	7.73±0.98	<0.0001
SBP (mmHg)	114.2±10.35	118.55±12.01	0.03
DBP (mmHg)	71.3±4.64	76.55±6.99	0.0003
HDL-C (mmol/L)	1.2±0.31	1.3±0.34	0.11
LDL-C (mmol/L)	1.8±0.69	3.43±0.80	<0.0001
TG (mmol/L)	1.6±0.59	1.7 ±0.87	0.46
TC (mmol/L)	3.9±0.91	4.95±1.01	<0.0001

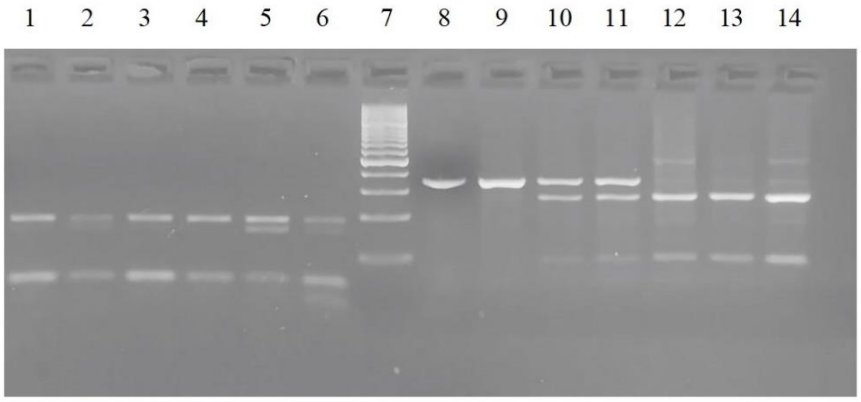
In both T2DM cases and controls, HWE analysis was performed using the rs7903146 and rs2237892 SNPs. The genotype distribution for the rs7903146 SNP differed between cases ($x^2=9.6$; $p=0.001$) and controls ($x^2=8.3$; $p=0.003$), as did the genotype distribution for the rs2237892 SNP in T2DM ($x^2=5.33$; $p=0.020$) and control patients ($x^2=0.94$;

p=0.004). Table 2 defines the details of HWE analysis.

The two potential genotype loci of rs7903146 polymorphism in the *TCF7L2* gene and rs2237892 polymorphism in the *KCNQ1* gene are examined by PCR-RFLP analysis in T2DM and controls in this study. The 188bp (TT genotype) PCR product was digested with *RsaI* restriction enzyme to provide band products of 159/29bp for the CC genotype and 188/159/29bp for the CT genotype (Fig. 1). The genotype frequencies of CC, CT, and TT were reported to be 75%, 16.7%, and 8.3% in T2DM cases (Fig. 2), and 93.3%, 5%, and 1.7% in control groups. The C and T alleles were found to be 0.83 and 0.17 in T2DM patients, respectively, and 0.95 and 0.05 in controls. T2DM cases and controls were found to have a significant association based on genotype (OR-4.14; 95%CI: 1.07-15.98; p=0.02), dominant model (OR-4.66; 95%CI: 1.44-15.04; p=0.005), and allele frequencies (OR-4.60; 95%CI: 1.66-12.70; p=0.001). Table 3 represents genotype and allele frequency information.

Table 2: HWE analysis for rs7903146 and rs2237892 SNP

rs7903146	Controls (n=60)	T2DM Cases (n=60)
C allele	115 (0.95%)	100 (0.83%)
T allele	05 (0.05%)	20 (0.17%)
HWE	0.04	0.17
X^2	8.3	9.6
P-values	0.003	0.001
rs2237892	Controls (n=60)	T2DM Cases (n=60)
C allele	119 (98.3%)	114 (95.1%)
T allele	01 (1.7%)	06 (4.9%)
HWE	0.01	0.05
X^2	0.94	5.33
P-values	0.004	0.020



Lane 1,3-4: TT genotype for rs7903146 polymorphism
Lane 2,5-6: CT genotype for rs7903146 polymorphism
Lane 7: 100bp DNA marker
Lane 8-9: TT genotype for rs2237892 polymorphism
Lane 10-11: CT genotype for is 2237892 polymorphism
Lane 12-13: CC genotype for rs2237892 polymorphism

Fig. 1: Purified digested products were loaded onto a 3% agarose gel with a 100bp DNA marker

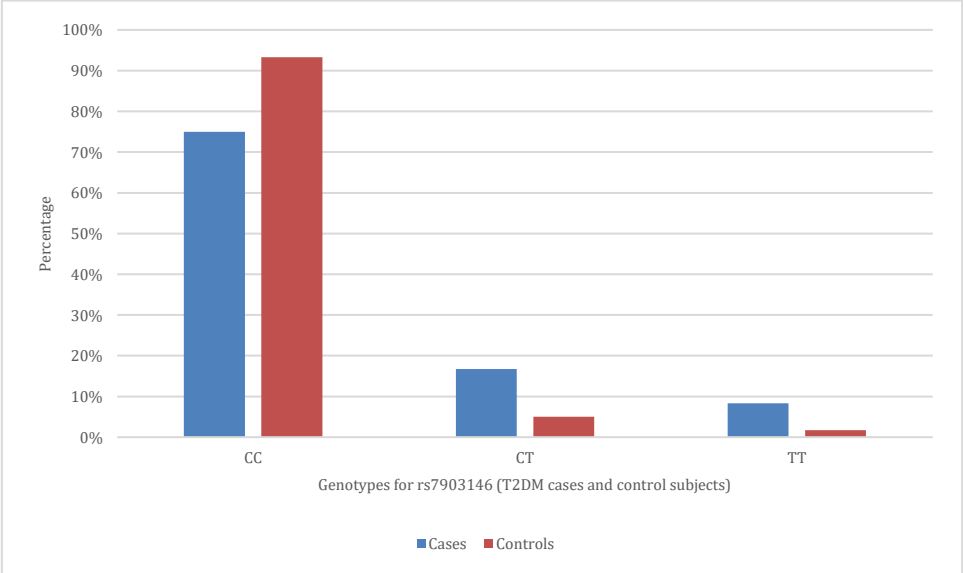


Fig. 2: Genotype frequencies for rs7903146 SNP in T2DM cases and control subjects

Table 3: Genotype frequencies for controls subjects and in T2DM cases and rs7903146 SNP

Genotype	Controls (n=60)	T2DM Cases (n=60)	OR (95%CI)	P-value
CC	56 (93.3%)	45 (75%)	-	-
CT	03 (5.0%)	10 (16.7%)	4.14 (1.07-15.98)	0.02
TT	01 (1.7%)	05 (8.3%)	6.22 (0.70-55.18)	0.06
TT+CT vs CC	04 (6.7%)	15 (25%)	4.66 (1.44-15.04)	0.005
C allele	115 (0.95%)	100 (0.83%)	-	-
T allele	05 (0.05%)	20 (0.17%)	4.6 (1.66-12.7)	0.001

Fig. 1 illustrates the three genotypes obtained in the rs2237892 SNP. The CC genotype was digested with the *HpaII* restriction enzyme, which cut the 354bp (TT genotype) PCR product into 269 and 85bp (CC genotype), and the combination of 354, 269, and 85bp shows heterozygous (CT genotypes) (Fig. 1). C and T allele frequencies were found to be 95.1 and 4.9 in T2DM patients, respectively, and 98.3 and 1.7 in controls. T2DM cases had genotype frequencies of 91.6%, 6.7%, and 1.7% (Fig. 3) and 98.3%, 1.7%, and 0%. In this study, none of the genotypes (OR-4.29; 95%CI: 0.46-39.58; p=0.16; OR-3.21; 95%CI: 0.12-80.6; p=0.45) and allele frequencies (OR-6.26; 95%CI: 0.74-52.83; p=0.055) confirmed the significant association. The genotype and allele frequencies in T2DM cases and controls with the rs2237892 SNP are shown in Table 4.

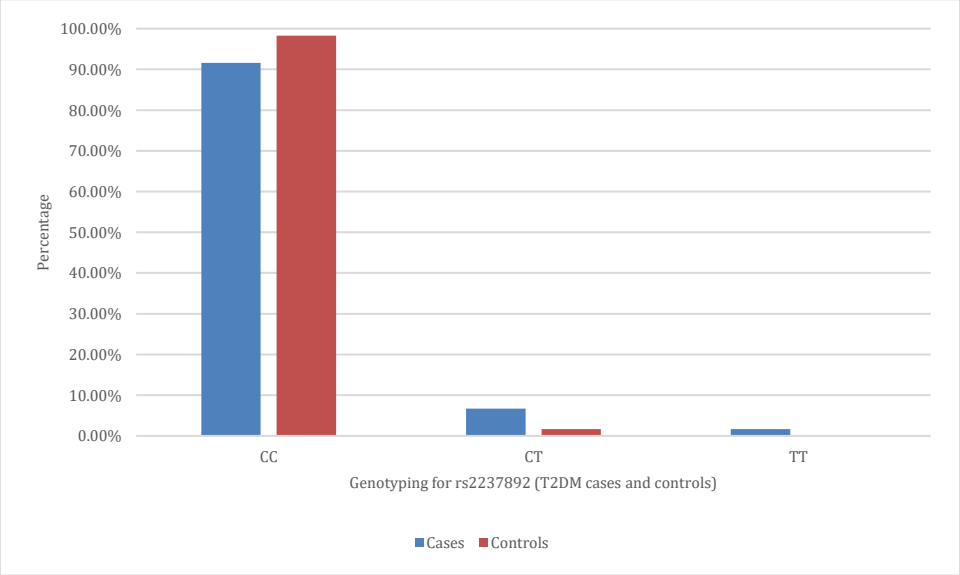


Fig. 3: Genotype frequency distribution in the rs2237892 SNP among T2DM patients and controls

Table 4: Genotype and allele frequencies between rs2237892 SNP in T2DM cases

Genotype	Controls (n=60)	T2DM Cases (n=60)	OR (95%CI)	P-value
CC	59 (98.3%)	55 (91.6%)	-	-
CT	01 (1.7%)	04 (6.7%)	4.29 (0.46-39.58)	0.16
TT	00 (0%)	01 (1.7%)	3.21 (0.12-80.6)	0.45
TT+CT vs CC	01 (1.7%)	05 (8.4%)	5.36 (0.60-47.36)	0.09
C allele	119 (98.3%)	114 (95.1%)	-	-
T allele	01 (1.7%)	06 (4.9%)	6.26 (0.74-52.83)	0.055

4. Discussion

T2DM is defined by the dysregulation of carbohydrate, lipid, and protein metabolism, which leads to either individual or combined insulin secretion or insulin resistance (DeFronzo et al., 2015). T2DM, which affects more than 90% of diabetics, is a combination of hereditary and environmental factors. There is a kind of dyslipidemia frequent in diabetics termed diabetic dyslipidemia, which is marked by low HDL levels and elevated TG levels. This pattern is most commonly associated with type 2 diabetes and may be a manageable risk factor for cardiovascular disease in the future. T2DM is a hereditary disease that affects people of different ethnicities in different ways (Elqadi et al., 2021). In our study, T2DM patients have normal levels of lipid profile and were found to be associated with LDLC and TC (p<0.0001), when compared with controls. The current study confirmed that T2DM patients had elevated BMI, weight, FBS, Hb1Ac, and HTN levels (p<0.05). Both Allele and genotype analysis confirmed the positive association with rs7903146 SNP (OR-4.14; 95%CI: 1.07-15.98; p=0.02; OR-4.66; 95%CI: 1.44-15.04; p=0.005; OR-4.60; 95%CI: 1.66-12.70; p=0.001) and negative association with rs2237892 SNP (OR-4.29; 95%CI: 0.46-39.58; p=0.16; OR-3.21; 95%CI: 0.12-80.6; p=0.45; OR-6.26; 95%CI: 0.74-52.83; p=0.055).

TCF7L2 was commonly viewed as a susceptibility gene for T2DM in people of various ethnicities. Grant et al. (2006) discovered the DG10S478 microsatellite marker in Icelandic people, which was substantially associated with T2DM (Grant et al., 2006). The

rs7903146 SNP in an Intron 3 (IVS3C>T) is related to T2DM and may function through decreased production of glucagon-like peptide 1, which is activated more by fat than carbohydrate ingestion. There is 215.9 kb of *TCF7L2* on chromosome 10q25. In T2DM genetic susceptibility testing, it is regarded as the most important gene. *TCF7L2* has been shown to be essential for the formation of the pancreas and islets during embryonic growth as a crucial transcriptional regulator of glucose metabolism via the Wnt signaling pathway (Khan et al., 2015a). Numerous populations studies have found rs7903146 is linked to an increased risk of developing T2DM (Syamsurizal et al., 2019; Shokouhi et al., 2014; Guewo-Fokeng et al., 2015) including meta-analysis studies (Ding et al., 2018; Hussain et al., 2014; Liu et al., 2015).

TCF7L2 (rs7903146) and *KCNQ1* (rs2237892) have been linked to mostly impaired β-cell function. Insulin-producing cells also express *KCNQ1*. The selective inhibitor chromanol 293B improves insulin secretion in INS-1 cells by inhibiting *KCNQ1* channel activity, whereas overexpression of *KCNQ1* in MIN6 cells dramatically impairs insulin secretion in response to glucose, pyruvate, or tolbutamide. A *KCNQ1* gene SNP has been linked to the development of diabetes in Asians based on GWAS. Susceptibility to T2DM has been linked to an SNP in the *KCNQ1* gene, which is found in adipose tissue. *KCNQ1* has several genetic variants, including three major SNPs located in the intron 15 of *KCNQ1* (rs2237892, rs2237895, and rs2237897). T2DM and decreased insulin secretion were found to be related to these polymorphisms in a variety of groups, including Asians, Europeans, and Native Americans, according to several GWAS studies (Zhang et al., 2015; Khan et al., 2015b). Our study results showed a negative association with rs2237892 SNP and were in agreement with other global studies (Erfani et al., 2020; Turki et al., 2012). Other studies were found to be positively associated (Yu et al., 2020; Yasuda et al., 2008; Chen et al., 2010). A limited meta-analysis was carried out between rs2237892 SNP and T2DM (Jiang et al., 2021, Sun et al., 2012).

The strength of this current study was to involve Saudi nationalities and we have screened rs7903146 and rs2237892 SNPs which plays a major role in T2DM. Involving 60 T2DM cases/60 controls was the major limitation of this study. Missing validation through Sanger sequencing analysis was one of the limitations of this study.

In conclusion, our study confirms rs7903146 SNP is associated with T2DM and rs223892 SNP was not found a positive correlation in our study. Future studies should be conducted with a large sample size and multiple SNPs.

Compliance with ethical standards

IRB details

The ethical grant for this study was obtained from Institutional Review Board and a patient-informed consent form was obtained for all subjects who participated in this study. In this study, we have excluded the participants who weren't signed the consent form. This study was conducted in accordance with the principles outlined in the Helsinki Declaration. This is a case-control study.

Conflict of interest

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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