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Original article

Amino-acid amendment of Arginine-325-Tryptophan in rs13266634 genetic polymorphism studies of the *SLC30A8* gene with type 2 diabetes-mellitus patients featuring a positive family history in the Saudi population

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ABSTRACT

Type 2 diabetes mellitus (T2DM) is a complex metabolic disorder with chronic hyperglycemia. Genome-wide association studies (GWAS) have identified many genes and, among them, solute carrier family 30 member 8 (SLC30A8) was one of the important genes linked to the development of T2DM risk. The relationship between T2DM and the SLC30A8 gene is linked through zinc, which plays a key role in the storage and secretion of insulin. The rs13266634 polymorphism includes a strong genetic association in case-control and *meta*-analysis studies of the global population. The aim of this current study was to scrutinize the genetic relationship between the rs13266634 polymorphism in the *SLC30A8* gene with T2DM subjects selected with a family history in the Saudi population. This study involved 120 cases of diagnosed T2DM and 120 confirmed healthy controls that were recruited to screen rs13266634 polymorphisms through a genotyping analysis followed by PCR and RFLP analysis. Baseline characteristics between cases and controls have been evaluated with Student's *t*-test. The study results confirmed the genetic association between the allele ($p = 0.001$), genotypes (CT = 0.005 and TT = 0.03), and various genetic patterns of inheritance ($p = 0.001$ and $p = 0.02$). Both analysis of variance (ANOVA) and binary logistic regression analysis revealed non-signiifcant association with T2DM cases and biochemical parameters ($p > 0.05$). In conclusion, the current results have confirmed the strong genetic association between T2DM cases and controls in the Saudi population with rs13266634 polymorphisms of the *SLC30A8* gene.

1. Introduction

Diabetes mellitus (DM) is characterized by chronic hyperglycemia and impaired carbohydrates, lipid and protein metabolism owing to complete or partial incompetence of insulin secretion or insulin action (Nauck and Meier, 2020). Globally, half a billion people are afflicted with diabetes and by 2045, the International Diabetes Federation cautioned that figure will reach 693 million (Zhang et al., 2020). Among different modes of diabetes, type 2 diabetes mellitus (T2DM) is a very common form of the disease that affects the age of onset and has been predicted as a heterogenous group of metabolic and multifactorial disorders (Khan et al., 2019). T2DM is growing epidemic worldwide and is associated with serious complications, which decreases lifespan and quality of life (Blasco-Blasco et al., 2020). The prevalence of adult-onset diabetes or T2DM was found to be 382 million in 2013, and by 2035, the disease will be highly susceptible in 592 million individuals across 130 countries (Athyros et al., 2020). T2DM disease is characterized by chronic hyperglycemia, which increases the risk of cardiovascular disease (Palella et al., 2020).

Obesity is a key aspect in the progression of T2DM, which is aligned with several life-threatening diseases, including gestational diabetes mellitus (GDM), heart disease, hypertension and cancer. Obesity and T2DM originates according to similar criteria for metabolic disorders, illustrating very strong family influences (Sheikhpour et al., 2020). Additionally, T2DM and GDM share similar pathophysiological characteristics of diabetes (Khan et al., 2019). Moreover, genetics and environmental factors play a vital role in disease progression (Khan et al., 2015a). In terms of β-cell

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activity, the mainstream genes involved play a major part and genetic polymorphisms that affect important proteins involved in glucose metabolism and insulin secretion can also impact susceptibility to T2DM (Witka et al., 2019). Genome-wide association studies (GWAS) have recognized multiple novel susceptibility genes for T2DM. In addition to GWAS, linkage analysis, candidate gene approach along with case-control and hospital-based studies have confirmed the various genes and variants that lead to susceptibility to T2DM (Khan et al., 2015b). In excess of one million single nucleotide polymorphisms (SNPs) have been identified using GWAS and high-throughput technology, which can overcome the limitations of the candidate gene approach (Mtiraoui et al., 2012). The solute carrier member 30, zinc transporter, member 8 (SLC30A8) gene was identified as an rs13266634 polymorphism in the first GWAS study of T2DM in the French population (Sladek et al., 2007). Zinc is an important component of insulin storage and secretion that is presumed to be a critical element underlying the insulin secretion mechanism and may attenuate insulin secretion. SLC30A8 encrypts the zinc carrier protein component-8, which contains eight exons and 369 amino acids, and is considered to be the β-cell zinc homeostasis regulator. The rs13266634 polymorphism is a non-synonymous SNP that causes an amino acid transition from arginine (R/C) to tryptophan (W/T) at position 325. *SLC30A8* gene expression significantly increases in T2DM pancreatic islets in patients with CT/TT genotypes. The gene expression levels in *SLC30A8* in pancreatic islets of T2DM patients carrying a risk allele is increased by approximately 2.5-fold compared to a non-diabetic control group. The TT genotypes with rs13266634 polymorphisms have the maximum level of gene expression (Faghih et al., 2014, Khan et al., 2015a, Khan et al., 2019, Khan et al., 2015b). Similar polymorphisms have been replicated in numerous case-control studies in T2DM throughout the globe and, based on case-control studies, *meta*-analysis studies have also been published. However, no full-fledged investigation has been tied to the Saudi population with a minimum three-digit sample size, which is the basic criteria for carrying out a case-control study either in T2DM cases or healthy controls. Therefore, the present study was designed to investigate the rs13266634 polymorphism in the *SLC30A8* gene in diagnosed T2DM subjects with a family history of diabetes in the Saudi population

2. Materials and methods

2.1. Sample selection

In this case-control study, 120 T2DM cases and 120 healthy controls were recruited from outpatient clinics in various regions of primary health clinics in the capital city of Saudi Arabia. An ethical grant (E-19-3694) was obtained from the institutional review board (IRB) at the College of Medicine at King Saud University (KSU). This study was in full compliance with Helsinki Declaration standards. The inclusion criteria for T2DM cases were based on World Health Organization (WHO) guidelines (126 mg/dL or >7.0 mmol/L) within Saudi nationals. The exclusion criteria for T2DM cases were based on Al-Daghri et al. (2012). The inclusion criteria of healthy control subjects had normal glucose tolerance (FBG < 7.0 mmol/L) without any family history of any form of diabetes within the family pedigree. The exclusion criteria were patients confirmed with diabetes (FBG > 7.0 mmol/L).

2.2. Anthropometric measurements

Age was recorded in years and gender was documented either as male or female. The details surrounding body mass index (BMI) were obtained based on the measurement of height in centimeters (cms) and weight in kilograms (kg). A validated mercurial sphygmomanometer was used to record the values of hypertension (HTN) of each participant one half-hour after completion of rest. Finally, waist and hip ratios were also determined for all participants.

2.3. Biochemical measurements:

A total of 3 mL of coagulant blood was collected and serum was used to measure fasting blood glucose (FBG) values; which were obtained based on WHO criteria and lipid-profile parameters, such as total-cholesterol (TC), high-density lipoprotein cholesterol (HDL-c), low-density lipoprotein cholesterol (LDL-c) and triglycerides (TG).

2.4. Molecular analysis

Using a Norgen DNA extraction kit (Norgen Biotec Corp., Canada), genomic DNA was extracted with 2 mL of peripheral blood collected in an EDTA vacutainer on the basis of the kit protocol. A NanoDrop Spectrophotometer was used to measure the concentration of genomic DNA. Both from T2DM and healthy controls, DNA samples were stored at −80 °C until genotyping was performed according to a polymerase chain reaction (PCR). Complete molecular analysis was carried out at the male campus of the Department of Clinical Laboratory Sciences, College of Applied Medical Sciences at the KSU premises of the genetics laboratory (G-141/1).

2.5. Amendment of amino acids in the R325W mutation of the SLC30A8 gene

The rs13266634 polymorphism was selected from the genes involved in T2DM recognized by GWAS. Genotyping was performed with PCR and then followed with a restriction fragment-length polymorphism (PCR-RFLP) analysis. Initially, PCR was carried out for a 50-μL reaction involving 50–100 ng of genomic DNA, 10 pmoles of +/- primers, Norgen master mix including 10x buffer, 25 mM of $MgCl_2$, 0.5 mM dNTPs and 10 units of Taq DNA polymerase and distilled water. PCR was conducted with initial denaturation (95 °C-5 min), denaturation (95 °C-30 s), annealing (60 °C-30 s), extension (72 °C-45 s) and final extension for 72 °C-5 min holding at 4 °C after the completion of reactions. PCR analysis was followed for 35 cycles in a thermal cycler. Sense and antisense sequences of the primers were adapted from Khan et al. (Khan et al., 2015a).

Next, the Hpa*II* restriction enzyme was used to digest the PCR products to reach the C$^{\downarrow}$CGG sequence to cut the amendment of the nucleotide sequence from C-T i.e., C$^{\downarrow}$CGG to C$^{\downarrow}$TGG (Fig. 1). A total of 15 μl of PCR product was employed for the digestion along with the 5 μl of distilled water, 4 μl of 10x buffer and 1 μl of 10 U/μL of Hpa*II* restriction enzyme (Thermo Fisher Scientific, USA), incubated at 37 °C for a couple of hours. Both the undigested and digested PCR products were run on 2% and 3% horizontal agarose gel electrophoresis; at 100 V, 12 W, 90 mA for a minimum of 60 min and maximum of 100 min stained with ethidium bromide and visualized on a UV transilluminator. Table 1 lists the primer sequences and SNP data in detail. Fig. 2 consists of undigested and digested band sizes of the rs13266634 polymorphism in the *SLC30A8* gene.

2.6. Validation

Sanger sequencing analysis was performed for 10% (12 samples of T2DM cases and 12 samples of healthy controls) subjects. A 256-bp primer sequence was selected for sequencing using standard dideoxy terminal chemistry and capillary electrophoresis method(s).

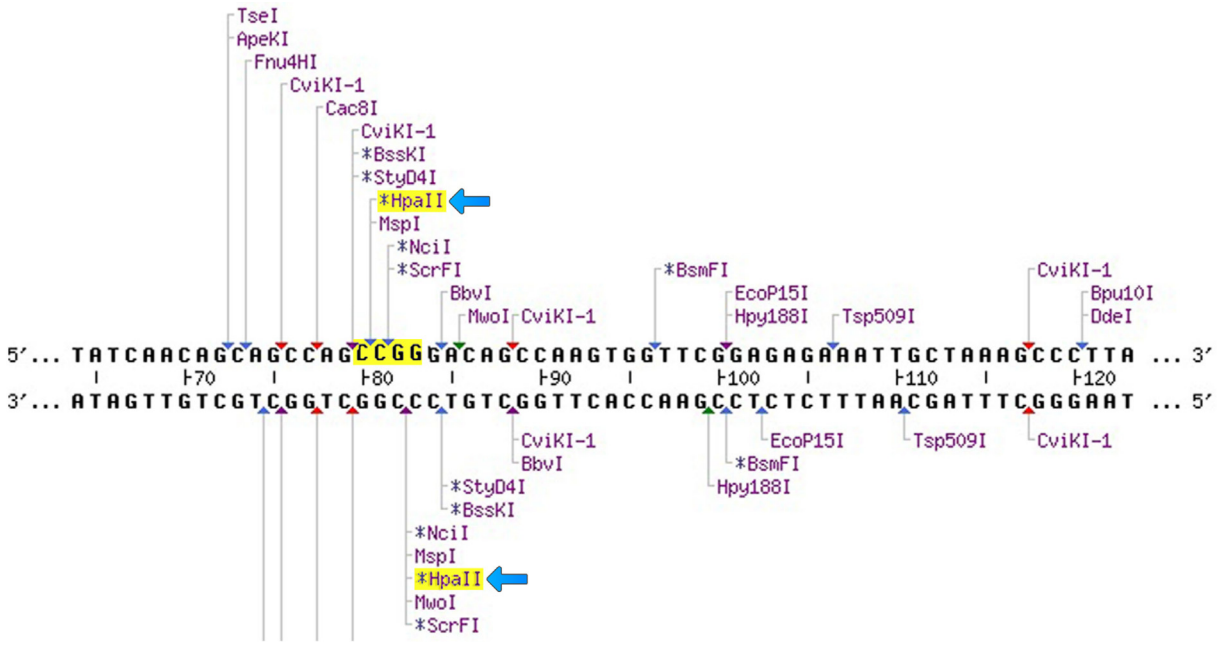

Fig. 1. Representation of the presence of the Hpa*II* restriction enzyme within the primer sequence used in this study.

Table 1
Details of single genetic polymorphism used in this study with T2DM subjects.

S. No	SNPedia	Orientation
1	Gene	SLC30A8
2	Reference sequence number	rs13266634
3	Mutation type	Non-synonymous single nucleotide polymorphism
4	Amino acid substitution	R325W or Arg325Trp
5	Single nucleotide polymorphism	C-**T**
6	Molecular region in the SLC30A8 gene	Exon-8
7	Human chromosome region	Chromosome-8 quinine region of 24.11 (8q24.11)
8	5′-3′ Primer sequence	F: GAAGTTGGAGTCAGAGCAGTC
9	3′-5′ Primer sequence	R: TGGCCTGTCAAATTTGGGAA
10	Band Size of the PCR product	256 bp
11	Restriction enzyme	*Hpa*II (G$^{\downarrow}$GCC)
12	Substitution of Nucleotide band size	176 bp
13	Position	117,172,544
14	Digested PCR products	CC: 176/80 bp; CT-256/176/80 bp and TT-256 bp
15	Condition	Type 2 Diabetes Mellitus
16	Organism	Homosapiens

Bidirectional sequencing analysis for PCR was carried out using a big-dye terminator for the amplified and purified products. DNA sequencing analysis was carried out in both forward and reverse sequences (Fig. 3).

2.7. Statistical data analysis

The Statistical Packages for Social Sciences (SPSS) software, version 25.0, for Windows (IBM, Chicago, USA) was utilized for statistical analysis. Means (M) and standard deviations (SD) are reported as continuous variables, while categorical variables are represented as percentages (%). The intergroup significance between T2DM cases and control subjects were evaluated by Student's *t*-test (continuous) and Chi-square test (categorical variables) in Table 2. Hardy-Weinberg equilibrium (HWE) was compared with one degree of freedom using the Chi-square test. Genotype and allele frequencies, including different numerous modes of inheritances (dominant, co-dominant and recessive) were performed and the results are presented in Table 3 with odds ratios (OR) and 95% confidence intervals (CI) (Das et al., 2017). The significance of one-way analysis of variance (ANOVA) and Chi-square tests were determined and the results are found in Table 4 as appropriate for T2DM cases. Binary logistic regression analysis was carried out with T2DM genotypes and lipid-profile parameters listed in Table-5. All P-values were two-sided and a p-value < 0.05 was considered as a statistically significant association between case-control groups

3. Results

3.1. Baseline characteristics of participant subjects

Anthropometric, biochemical and clinical data for T2DM cases and healthy controls are shown in Table 2. The mean age of the T2DM cases (55.80 ± 11.09) and healthy controls (46.25 ± 7.86) were significantly associated ($p < 0.0001$). Anthropometric measurements, such as age, weight, BMI, waist, hip, SBP and DBP were strongly associated in T2DM subjects compared to healthy controls ($p < 0.0001$). Biochemical parameters, like FBG and lipid profiles, such as that for TC, TG and HDL-c are significantly associated ($p < 0.05$) and not with LDL-c ($p = 0.70$). All T2DM cases had a family history and all healthy controls had no family history ($P = 1.00$).

3.2. HWE and genotyping analysis

Genotype frequency distributions of the rs13266634 polymorphism in the *SLC30A8* gene within the control group obeyed the HWE ($p > 0.05$). Allele and genotype frequencies between T2DM patients and control groups in the rs13266634 polymorphism of the *SLC30A8* gene (Fig. 4) and their genetic mode of inheritances with the risk of T2DM are shown in Table 3. The frequencies of CC, CT and TT genotypes were 45.8%, 36.7% and 17.5% in T2DM patients, respectively, and 65.9%, 23.3% and 10.8% in healthy controls, respectively. Statistical analysis between T2DM cases and

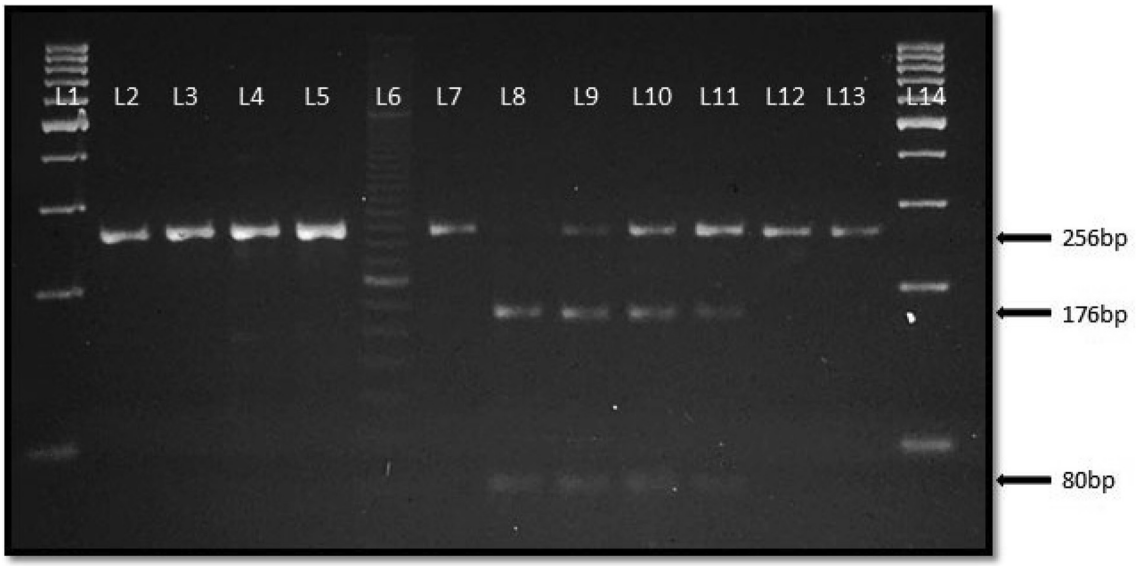

Fig. 2. Ethidium bromide-stained 3% agarose gel electrophoresis.

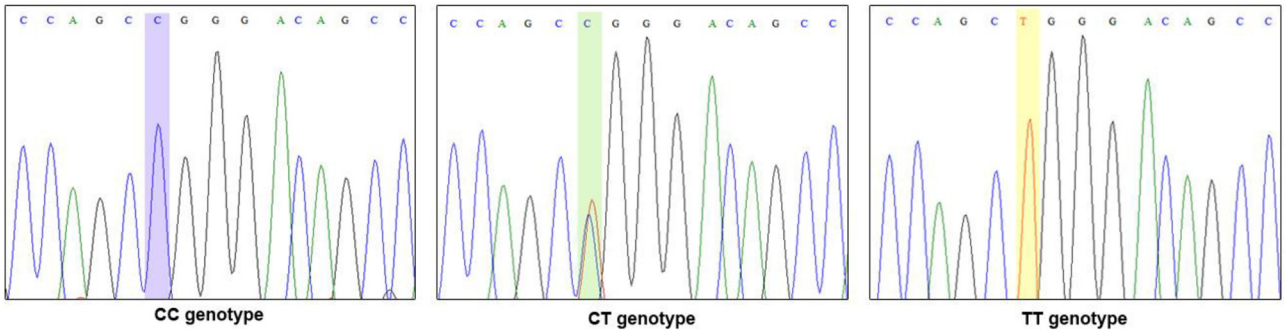

Fig. 3. Validation analysis of the rs13266634 polymorphism through Sanger sequencing.

Table 2
Baseline characteristics of diabetes and non-diabetic cases involved in this study.

Baseline characteristics	T2DM cases (n = 120)	Healthy Controls (n = 120)	P-value
Age (Years)	55.80 ± 11.09	46.25 ± 7.86	<0.0001
Gender (M: F)	1.32 ± 0.46	1.46 ± 0.50	0.024
Weight (kgs)	74.61 ± 12.60	70.05 ± 9.83	0.002
Height (cms)	163.23 ± 8.91	161.98 ± 8.67	0.27
BMI (kg/m^2)	28.19 ± 4.31	26.34 ± 2.94	<0.0001
Waist (cms)	90.83 ± 18.72	89.60 ± 18.22	0.60
Hip (cms)	123.94 ± 6.82	102.57 ± 20.79	<0.0001
SBP (mmHg)	124.69 ± 11.11	114.79 ± 7.90	0.001
DBP (mmHg)	78.43 ± 6.67	75.63 ± 6.10	0.001
FBG (mmol/L)	13.31 ± 5.33	5.23 ± 0.59	<0.0001
TG (mmol/L)	2.32 ± 1.88	1.65 ± 0.81	<0.0001
TC (mmol/L)	5.67 ± 1.23	5.20 ± 0.95	0.001
HDL-c (mmol/L)	0.88 ± 0.37	0.66 ± 0.25	<0.0001
LDL-c (mmol/L)	3.78 ± 0.80	3.82 ± 1.02	0.70
Family History	120 (100%)	00 (0%)	1.00

healthy control subjects exhibited signiifcant association with alleles (T vs. C: OR-1.92 (95% CI: 1.28–2.87; p = 0.001), genotypes (CC vs CT + TT: OR-2.27 (95%CI: 1.35–3.83; p = 0.001; CT vs. CC + TT: OR-1.90 (95% CI: 1.08–3.34); p = 0.02) and different inheritance patterns, such as heterozygous (CT vs. CC: OR-2.25 (95% CI: 1.25–4.05); p = 0.005) and homozygous variants (TT vs. CC: OR-2.32 (95%CI: 1.07–5.02); p = 0.03). However, the recessive mode of inheritance (TT vs. CC + CT: OR-1.74 (95% CI: 0.82–3.67); p = 0.13) was not statistically associated with this study.

3.3. Analysis of variance

One-way ANOVA was performed in T2DM cases and on rs13266634 polymorphism genotypes. Anthropometric measurements, biochemical parameters and clinical data were categorized as CC, CT and TT genotypes of the rs13266634 polymorphism in the *SLC30A8* gene. The genetic risk associated with the involved

Table 3
Genotype and allele frequency distribution between T2DM cases and healthy controls with the R325W polymorphism in the *SLC30A8* gene.

Mode of Inheritance	Genotype/Allele	T2DM cases (n = 120)	Controls (n = 120)	OR (95%CI)	Pvalue
Homozygous	CC genotype	55 (45.8%)	79 (65.9%)	Reference	Reference
Heterozygous	CT genotype	44 (36.7%)	28 (23.3%)	2.25 (1.25–4.05)	0.005
Homozygous variant	TT genotype	21 (17.5%)	13 (10.8%)	2.32 (1.07–5.02)	0.03
Dominant	CC vs CT + TT	65 (54.2%)	41 (34.1%)	2.27 (1.35–3.83)	0.001
Co-Dominant	CT vs CC + TT	44 (36.7%)	28 (23.3%)	1.90 (1.08–3.34)	0.02
Recessive	TT vs CC + CT	21 (17.5%)	13 (10.8%)	1.74 (0.82–3.67)	0.13
Homozygous Allele	C allele	154 (64.2%)	186 (77.5%)	Reference	Reference
Risk Allele	T allele	86 (35.8%)	54 (22.5%)	1.92 (1.28–2.87)	0.001

Table 4
ANOVA analysis between the R325W genotypes in the *SLC30A8* gene.

Baseline characteristics	CC (n = 55)	CT (n = 44)	TT (n = 21)	P-value
Age (Years)	55.95 ± 11.10	55.07 ± 11.45	56.95 ± 10.67	0.810
Gender (M: F)	1.35 ± 0.48	1.30 ± 0.46	1.29 ± 0.46	0.825
Weight (kgs)	68.64 ± 11.13	70.72 ± 8.48	72.33 ± 7.87	0.292
Height (cms)	162.55 ± 10.24	163.33 ± 7.85	164.81 ± 7.24	0.614
BMI (kg/m^2)	26.18 ± 3.41	26.33 ± 2.48	26.80 ± 2.54	0.711
Waist (cms)	90.17 ± 17.83	90.53 ± 18.05	90.83 ± 18.72	0.816
Hip (cms)	23.28 ± 6.28	24.64 ± 7.89	23.94 ± 6.82	0.609
SBP (mmHg)	123.20 ± 10.71	125.43 ± 10.59	127.05 ± 13.07	0.348
DBP (mmHg)	77.95 ± 6.16	78.16 ± 6.53	80.29 ± 8.10	0.373
FBG (mmol/L)	13.66 ± 5.48	12.68 ± 5.53	13.68 ± 4.56	0.626
TG (mmol/L)	1.69 ± 0.92	1.73 ± 0.70	1.36 ± 0.66	0.200
TC (mmol/L)	4.87 ± 0.89	5.24 ± 0.76	5.29 ± 1.08	0.223
HDL-c (mmol/L)	0.65 ± 0.26	0.64 ± 0.26	0.73 ± 0.18	0.446
LDL-c (mmol/L)	3.86 ± 0.85	3.80 ± 0.74	3.52 ± 0.74	0.258
Family History	55 (100%)	44 (100%)	21 (100%)	1.00

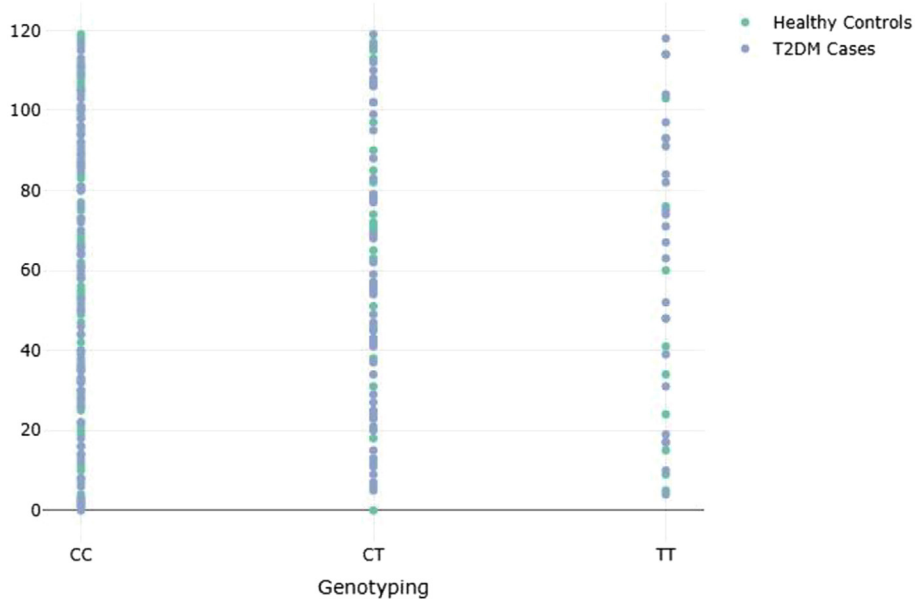

Fig. 4. Genotype frequencies between T2DM cases and Healthy controls in rs13266634 polymorphism.

underlying characteristics and genotypes was calculated and is seen in Table 4. Age (56.9 ± 10.6), weight (72.3 ± 7.8), height (164.8 ± 7.2), BMI (26.8 ± 2.54), waist (90.8 ± 18.7), SBP (127.05 ± 13.07), DBP (80.29 ± 8.1), FBG (13.6 ± 4.5), TC (5.29 ± 1.0) and HDL-c (0.73 ± 0.1) have been found to be high in TT genotypes. Hip (24.6 ± 7.8) and TG (1.7 ± 0.7) were high in CT genotypes while gender (1.3 ± 0.4) and LDL-c (3.8 ± 0.8) were high in CC genotypes. Statistical ANOVA for genotypes CC, CT and TT did not exhibit any statistical association ($p > 0.05$).

3.4. Binary logistic regression analysis

Binary logistic regression analysis was performed with CC, CT and TT genotypes on lipid-profile parameters, such as TC, TG, HDL-c and LDL-c. Fig. 5 describes the relationship with lipid profile and confirms the negative association with CC, CT and TT genotypes. No significant correlation was documented between the three genotypes and lipid-profile parameters ($p > 0.05$).

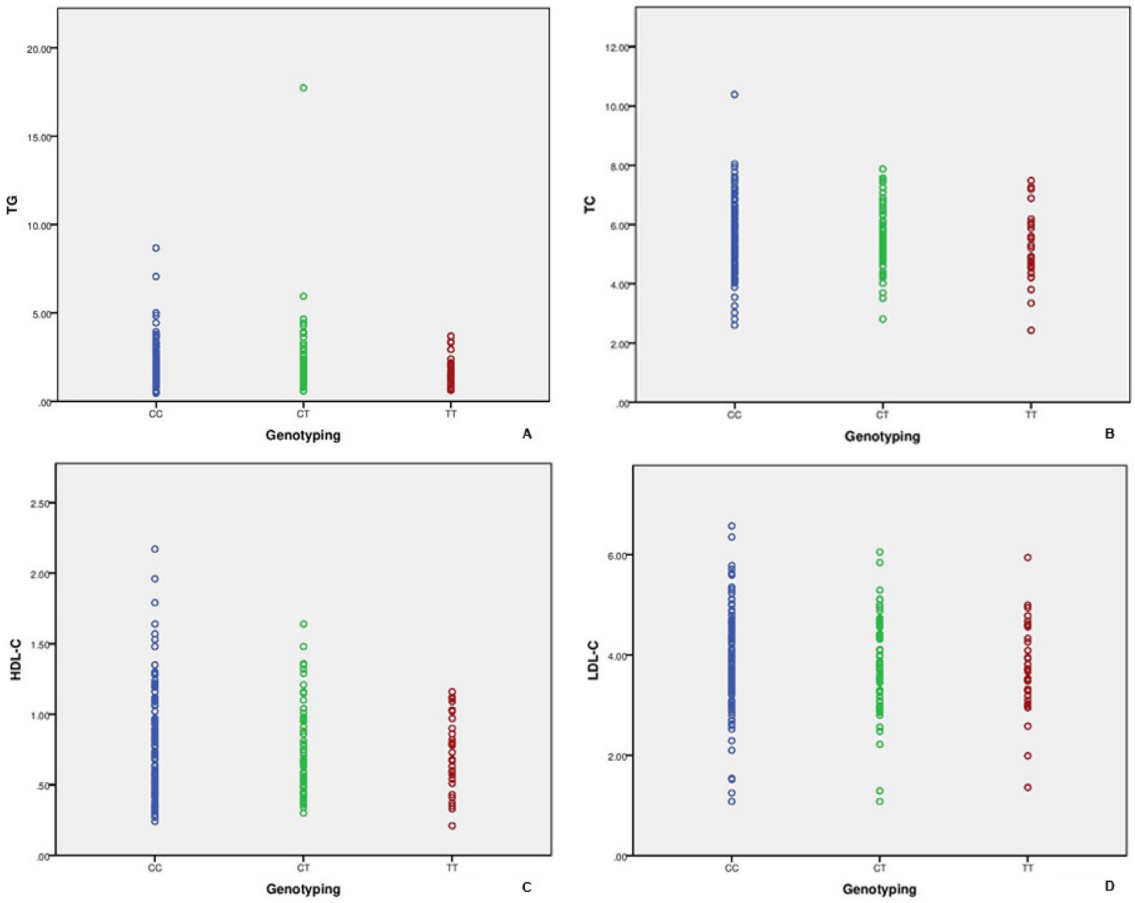

Fig. 5. Binary logistic regression analysis performed between lipid profile and genotyping.

4. Discussion

Diabetes is a polygenic metabolic condition that impairs an extremely complex interaction of genetic and environmental factors. Diabetes can lead to chronic complications following a deficiency in protein, fat and carbohydrate metabolism. The Kingdom of Saudi Arabia is one of the emerging countries in the Middle East region, where diabetes is recognized as a primary clinical and public health conundrum. Previous studies have revealed that 23.7% of Saudis are affected with diabetes, with a higher concentration in males compared to females (Alhomayani et al., 2020). However, based on an IDF report, the estimated cost of diabetes patients is expected to reach 592 million by 2035 (Wang et al., 2018). The economic and health burden of diabetes and its accompanied complications is substantial. Subsequently, the progression of diabetes is growing specifically in the era of obesity and diabetes in both children and young adults (Guan et al., 2020). Inherited factors play a leading role for human diseases along with the genes that remain poorly defined. Mutations or SNPs in any gene are unique. Mutations of any gene in pronounced form of diabetes can lead to the disease's formation. In the case of T2DM, numerous genes are associated with the development of the disease in humans. The advancement of T2DM disease can be attributed to the enhanced effects of combined or individual genetic factors with environmental exposure (Xiao et al., 2016). Enormous functional and non-functional SNPs implicated in the genetic basis of the pathogenesis of T2DM are massively applied in genome-wide linkage analysis, the candidate gene approach and GWAS (McCarthy and Zeggini, 2009).

The results of the current study demonstrate the genetic association between allele (p = 0.001), genotype (CT = 0.005 and TT = 0.03), as well as dominant (p = 0.001) and co-dominant (p = 002) mode of inheritances between T2DM cases and controls with the rs13266634 polymorphism in the *SLC30A8* gene. ANOVA analysis did not demonstrate the correlation between the rs13266634 genotypes or the anthropmetric and biochemical parameters involved in this study (p > 0.05). Multiple logistic regression analysis also failed to show the statistical association in T2DM cases (p > 0.05). One of the nominal reasons could be the limited sample size involved in this study. However, Al-Aama et al. (2019) conducted a pilot study in 46 T2DM subjects diagnosed in the Saudi population of the Jeddah region, along with 42 controls analyzed for rs13266634 and other PCR-specific SNPs, and they were followed by SNaPshot analysis that confirmed the negative association. The current study findings were not in agreement and one of the reasons for this is the large (n = 120) sample size of this work.

The rs13266634 polymorphism is carried across the global population and confirms the strong potential risk factors, statistical association (Soltanian et al., 2020, Yazdi et al., 2020) and non-significant associations (Kulkarni et al., 2016, Khan et al., 2015b) based upon ethnicities. The CT genotypes in our study group was found to be 36.7% in T2DM cases, which was similar in other ethnic groups, such as 38.2% and 38% (Cauchi et al., 2008a, 2008b). TT genotypes were documented to be 17.5% in our study and close frequencies were confirmed in the Chinese population (Han et al., 2010). Limited *meta*-analysis studies have been carried out with T2DM disease focusing on the rs13266634 polymorphism in the *SLC30A8* gene. Cheng et al. (Cheng et al., 2015) carried out *meta*-analysis studies between the rs13266634 polymorphism with T2DM and confirmed the probable important genetic factor in Asians and Europeans, yet failed to show the association in African

ethnicity. An updated *meta*-analysis study by Fan et al. (Fan et al., 2016) confirmed the significant association of T2DM with the rs13266634 polymorphism in the *SLC30A8* gene in Asian, European and African ethnic groups. Dong et al. (2018) also performed a *meta*-analysis study selecting T2DM patients afflicted with the rs13266634 polymorphism only in the Chinese population and as a potential risk factor.

In an Asian Indian population, Khan et al. (2019), Khan et al. (2015b), Khan et al. (2015a) performed a case-control study with three different forms of diabetes, including T2DM, post-transplant diabetes mellitus (PTDM) and GDM with the rs13266634 polymorphism, and their findings indicated there to be a non-significant association ($p = 0.74$) with T2DM, a nominal association ($p = 0.01$) with PTDM and a strong significant association ($p = 0.0003$) with GDM. One of the parallel relationships between T2DM, PTDM and GDM is shared by the similar pathophysiology and once the diabetic disease develops in any individual, it will last forever throughout their lives. Moreover, one of the anti-parallel relationships between these three forms of diabetes is T2DM develops based on the age of onset, PTDM develops after renal transplantation via immunosuppressive drugs and GDM develops only in women during the middle trimester of pregnancy based on the lack of glucose intolerance. One of the multi-factorial T2DM disease etiological factors emanates from defects in β-cell dysfunction. The *SLC30A8* gene is connected and expressed in pancreatic β-cells involved in the development of T2DM-specific diabetes by disrupting the function of β-cells and transporting zinc from the cytoplasm to insulin-secretary vesicles (Khan et al., 2019; Fan et al., 2016). Fig. 6 describes the relationship between diabetes with the *SLC30A8* gene as based on the aid of glucose metabolism, whereby glucose enters into β-cells and is converted into glucose-6-phosphate dehydrogenase, so its stimulus and its secretion is based on β-cell coupling. Further, glucose causes the closure of ATP-sensitive K^+ (K^{ATP}) channels through mitochondrial ATP production and an increased ATP-ADP ratio, eliciting action potentials that are associated with the opening of Ca^{+2} voltage-gated channels. The rise in $(Ca^{+2})_I$ within β-cells and insulin production is more or less constant, regardless of blood glucose levels. To-be-released Ca^{2+} is stored within vacuoles via exocytosis, which is primarily caused by food, mainly nutrition containing absorbable glucose. Blood glucose levels rise after digestion, the main trigger. Finally, insulin secretion is inhibited by the *SLC30A8* gene (Khan et al., 2015a, Khan et al., 2019, Khan et al., 2015b).

A family history of Diabetes is related to a number of metabolic disorders, which is a major risk factor for T2DM, which has a powerful genetic link, and if both parents have diabetes, individuals with a family history are at greater risk of developing the disease with a two-fold risk (Consortium, 2013). In this study, all T2DM patients had a family history of diabetes, which indicated that participants had a risk of manifesting T2DM and inheriting the disease within the family pedigree. The current study had certain strengths, such as: (i) opting for Saudi subjects as an ethnic group; and (ii) all subjects had a family history of diabetes. The limitations of this study were: (i) performing only single SNP analysis along with the limited sample size; (ii) not conducting protein measurement; and (iii) not establishing the lifestyle and physical activities of T2DM patients.

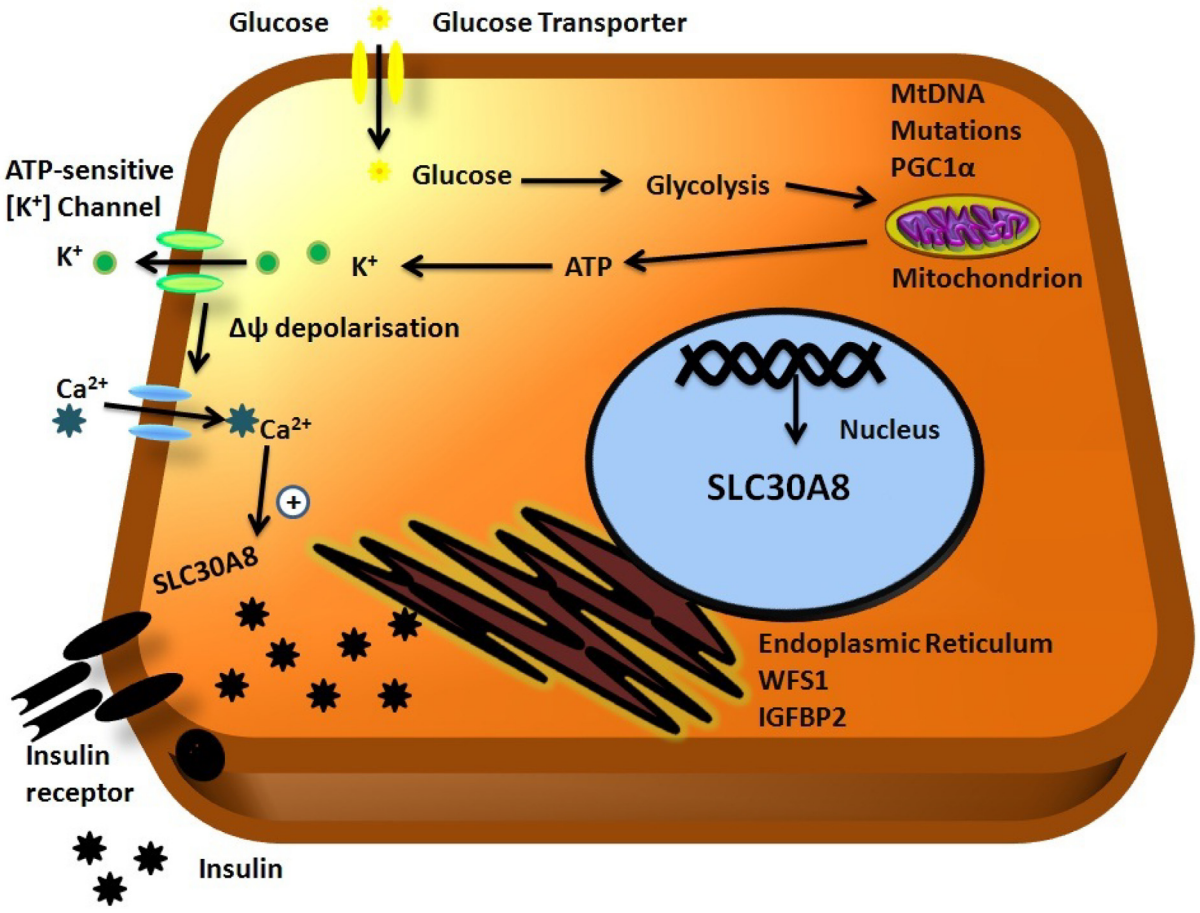

Fig. 6. Genetic relationship between the *SLC30A8* gene and diabetes.

5. Conclusion

In conclusion, the present study's results confirmed the genetic relationship with the rs13266634 polymorphism in the *SLC30A8* gene in T2DM cases in the Saudi population. However, this study was not in agreement with the prior study implemented in the Hejaz region featuring a low sample size. Future studies with a large sample size with an additional SNPs in the *SLC30A8* gene should be carried out. Additionally, this study recommends that the *meta*-analysis should be updated for rs13266634 in the *SLC30A8* gene as case-control studies have been documented in global T2DM ethnicities.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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