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Original article

Genetic polymorphism of HHEX rs1111875 in type 2 diabetes: Insights from a Jordanian case-control study

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Abstract. *Background and aim:* Type 2 diabetes (T2D) is a complex disease predisposed by both genetic and environmental factors. Many genes related to β-cell function, glucose regulation, and insulin secretion play crucial roles in T2D development. Human genome studies identified several genetic variations associated with T2D among different populations, including *HHEX* rs1111875 gene polymorphism. This study aims to investigate the association between *HHEX* (rs1111875) genetic polymorphism and the risk of T2D among the studied Jordanians (n= 200). *Methods:* This case-control study enrolled 100 patients with T2D, and 100 age and sex-matched healthy control groups fulfilled the inclusion criteria, aged between 33-66 years, with 50% males and 50% females in each group. Both groups were genotyped for rs1111875 polymorphisms using the Real-time PCR Allelic Discrimination Assay technique. *Results:* A significant association was observed between the *HHEX* genotypes (AG, AA) and the increased risk of T2D (p = 0.012). According to allele frequency, A allele of *HHEX* rs1111875 was strongly associated with increased risk of T2D by approximately twofold (Odd Ratio (OR)= 1.98, 95% Confidence Interval (CI)= 1.30-3.02, p= 0.002). Based on the dominant model, having at least one A allele increases T2D risk by threefold (OR= 3.63, 95% CI= 1.50-8.80, p = 0.003). This finding was confirmed in the overdominant model as the AG genotype increases T2D risk by twofold (OR= 2.88, 95% CI= 1.22-6.78, p-value= 0.014). *Conclusions: HHEX* rs1111875 A allele is significantly associated with increased T2D risk among Jordanians. (www.actabiomedica.it)

Key words: HHEX, rs1111875, polymorphism, type 2 diabetes, genetic association, Jordan, case-control study, type 2 diabetes mellitus, gene variance

Introduction

Type 2 Diabetes (T2D)

T2D is a complex metabolic disorder induced by environmental and genetic predisposing conditions that cause defective insulin secretion from pancreatic β-cells or inappropriate responsiveness to insulin by insulin-sensitive tissues, leading to hyperglycemia (1). According to the International Diabetes Federation (IDF), T2D affects 90% of diabetes cases globally, with a prevalence of 10.5% in 2021, and is predicted to reach 12.2% by 2045 among adults aged 20–79 (2). In Jordan, there was an increase of 14.3% in T2D prevalence between 1990 (14.0%) and 2020 (16.0%), and more increases of 28.8% are expected between 2020 (16.0%) and 2050 (20.6%), which implies that one out of five Jordanians will be diagnosed with diabetes by the year 2050 (3). Various modifiable and non-modifiable risk factors contribute to T2D development (4). Obesity, physical inactivity, smoking, diet, hypertension, and dyslipidemia are all modifiable factors, while, age, sex, and family history of diabetes are non-modifiable risk factors (5). Polymorphisms in genes affect proteins involved in glucose metabolism and insulin secretion, resulting in susceptibility toT2D (6).

Researchers have identified several T2D variants using GWAS (7). Around four hundred loci were identified as a susceptible risk for T2D (8), and around seventy loci positioned in and around the cyclin-dependent kinase inhibitor 2A/B (*CDKN2A/B)*, Cyclin-dependent kinase 5 (CDK5) regulatory subunit associated protein 1 like 1 (*CDKAL1*), solute carrier family 30 member 8 (*SLC30A8)* and hematopoietically expressed homeobox *(HHEX)* genes were associated with T2D (9).

HHEX gene

The proline-rich homeodomain protein (PRH)/HHEX is a transcription factor encoded by the *HHEX* gene, which consists of four exons and is located on chromosome 10q23.33 (Figure 1) (10). It spans 5.7 kb, encodes 270 amino acids, and is a member of the homeobox family of transcription factors involved in embryonic developmental processes(11). HHEX protein has three functional domains (12) that influence β-cell activity and development by activating the hepatocyte nuclear factor-1α protein (13). It is a target for the Wnt/β-catenin signaling pathway necessary for the development of the pancreas, liver, and blood vessels (14). Wnt proteins regulate glucose-stimulated insulin secretion and pancreatic proliferation (15).

HHEX gene is selectively expressed in δ-cells that secretes somatostatin (16). Somatostatin typically exerts paracrine inhibition of glucagon and insulin release from neighboring α and β-cells, respectively. Decreased somatostatin levels may result in disrupted inhibition, potentially leading to dysregulated insulin secretion and T2D development (17). *HHEX* SNPs were evaluated among patients with T2D (18). A meta-analysis study enrolled 162663 subjects and showed a significant association between *HHEX* SNPs and T2D (19). rs1111875 SNP located on the 3' flanking region of the *HHEX* gene on chromosome 10 in position 92703125 was highly associated withT2D (20). A significant association between the rs1111875 G allele and T2D risk was shown among the Tunisian population (21) but not

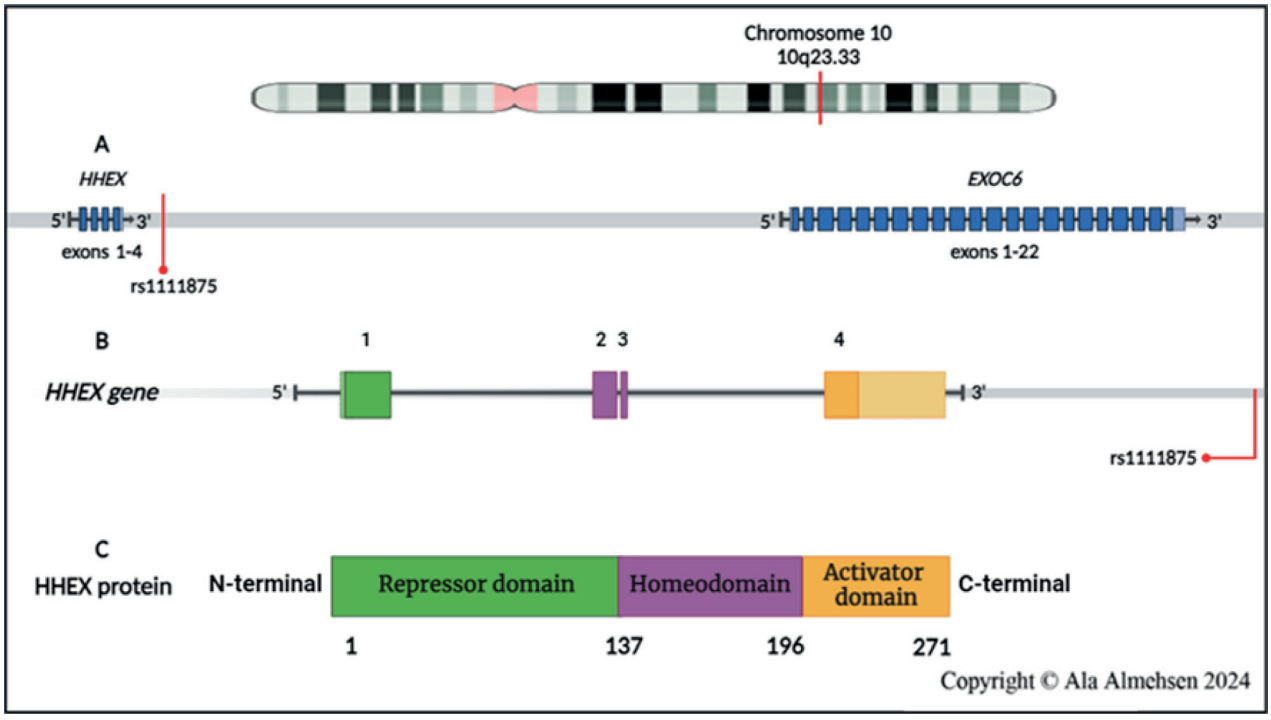

Figure 1. *HHEX* gene and rs1111875 polymorphism. (A) rs1111875 SNP located in the intergenic region between *HHEX* and exocyst complex component 6 (EXOC6) genes of the long arm q23.33 of Chromosome 10, (B) Exons of the *HHEX* gene and the location of rs1111875 SNP (C) the HHEX protein with three functional domains, the n-terminal repressing domain in green, the DNA binding homeodomain in purple, and C-terminal activating domain in orange.

among Indians (22) . European GWAS study showed an association between the rs1111875 GG polymorphism carriers and decreased pancreatic β-cells function (23). An association between rs1111875 and increased insulin resistance plays a significant role in T2D development within the Iranian (24) and Han Chinese populations (25). Many studies have been conducted on the association between *HHEX* gene polymorphism and T2D. However, these studies have conflicting outcomes. While many SNPs near the *HHEX* gene show associations, rs1111875 has consistently shown the strongest signal, making it a reliable marker for further study. There is no previous study investigating the association between *HHEX* (rs1111875) and the risk of T2D among Jordanians, so this case-control study was conducted to find out.

Material and methods

Study design and participants

This case-control study enrolled one hundred T2D patients recruited from the Diabetes and Endocrinology Clinic (February 2023 -July 2023) and one hundred age-sex-matched controls selected randomly from the Jordanian population attending Prince Hamza Hospital. Ethical approval was obtained from the institutional review board committee at Hashemite University (2022, ref no. 2200724). We informed all participants of the study objectives and completed consent forms. We also followed Good Clinical Practice based on the Declaration of Helsinki (Fortaleza, 2013) and Good Laboratory Practice when analyzing samples. Inclusion criteria included cases diagnosed with T2D according to the American Diabetes Association diagnostic guidelines: FBG ≥126 mg/dL and HbA1c ≥ 6.5. The control subjects had no history of diabetes (type 1 or type 2), confirmed by having HbA1c<5.7% and FBS<100 mg/dL, while those with a family history of T2D and females who had ever been diagnosed with gestational diabetes were excluded (Figure 2).

Data collection

Demographic data were collected by conducting a structured interview based on the questionnaire that was obtained from some questions in The National

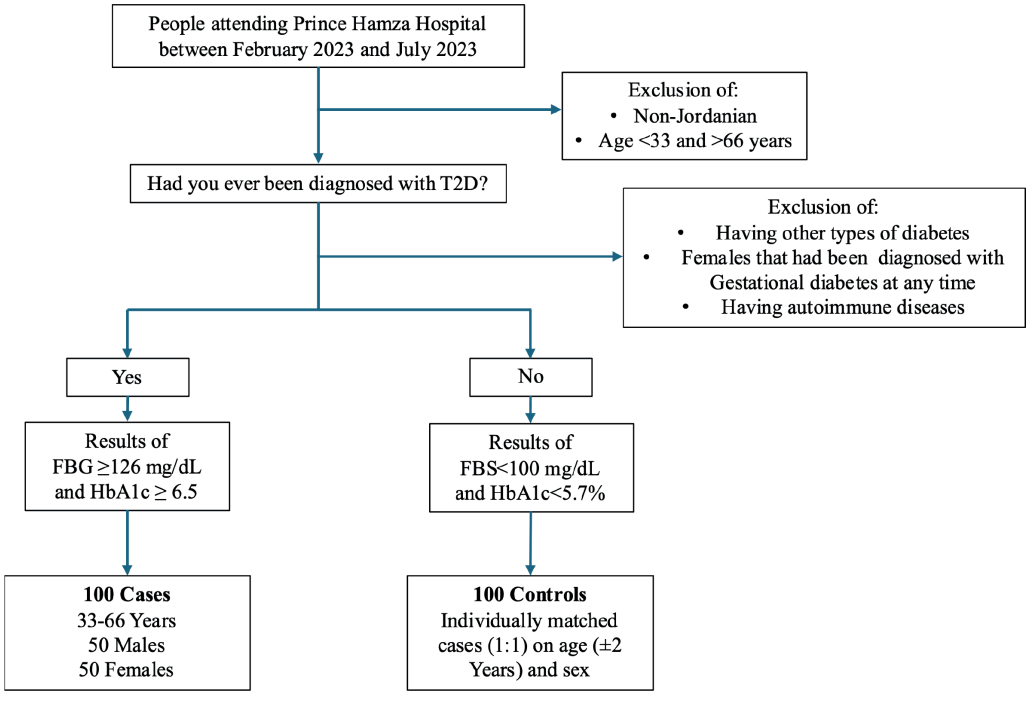

Figure 2. Inclusion and exclusion criteria chart

Survey of People with Diabetes (26), including sex, age, diabetic family history, smoking, heart disease, and dyslipidemia, It is important to acknowledge that the data on family history and smoking status were obtained through self-reporting by the participants. Several biomarkers and measures were selected to assess Selected biomarkers and measures assess obesity (WC, BMI), cardiovascular risk (SBP, DBP, TC, TG, HDL-C, LDL-C, VLDL-C), glycemic control (FBG, HbA1c), insulin production (C-peptide), and insulin dynamics (HOMA2-IR, HOMA2-%B, HOMA2-%S) in T2D. Weight in kilogram (kg) and height in centimeters (cm) were measured using an electronic balance and standard measuring tape, respectively. The BMI was calculated by applying the equation BMI (kg/m^2) = weight (kg)/height^2 (m). BMI results were considered underweight if BMI below 18.5 kg/m^2; normal weight if BMI ranges from 18.5 to 29.4 kg/m^2; overweight if BMI between 25 and 29.9 kg/m^2; and obese if BMI of 30 kg/m2 or higher. Waist circumference was recorded using the standard meter on the midpoint position between the lowest rib and the lateral iliac crest in centimeters (cm); having a waist circumference of more than 88 cm in females and more than 102 cm in males was considered a risk for T2D. Both systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured using a blood-pressure M3 device (Omron, Japan). Normal blood pressure (SBP/DBP) is below 120/80 mmHg (27).

Blood sample collection and processing

Two venous blood tubes were withdrawn for biochemical and molecular analysis. Plain tubes were centrifuged within an hour; the serum was used to measure biochemical tests. An ethylene diamine tetraacetic acid (EDTA) tube was used for the HbA1c test and DNA extraction, which was stored at -4°C until extraction. HbA1c was carried out using the Alinity c analyzer (Abbott, USA), while the C-peptide test was performed using the LIAISON® XL analyzer (Diasorin, Italy). FBG was measured using the spectrophotometry method, and the immunoassay method was used to measure HbA1c. The colorimetric method measured the TC, TG, HDL-C, and LDL-C lipid profiles. In contrast, VLDL-C was estimated using Martin's formula=TG/adjustable factor (28) using an LDL-C calculator. Fasting blood C-peptide was measured using a chemiluminescence immunoassay (CLIA) technique.

The Homeostatic Model Assessment version 2.2.3 (HOMA2) calculator was used to evaluate steady-state β-cell function (HOMA2-%B), insulin sensitivity (HOMA2-%S), and insulin resistance (HOMA2-IR) as percentages of a normal reference population using both FBG and fasting C-peptide levels (29). Auto calculations were performed for each test by embedding each sample result of FBG and fasting C-peptide in an Excel spreadsheet of the HOMA2 Calculator.

DNA extraction and concentration

DNA was extracted from peripheral blood using the QIAamp DNA Blood Mini Kit (QIAGEN, USA) by the QIAcube® machine (QIAGEN, USA). Samples were stored at -80 °C until used. Qubit™ 1X dsDNA HS Assay (Invitrogen, USA) was used to measure DNA quantities using Invitrogen™ Qubit™ 3.0 Fluorometer. DNA concentration was around 34.0 ng/μL for all samples.

SNPs primer and probe designing

SNP Selection was guided by literature for T2D from the National Center for Biotechnology Information (NCBI). For real-time PCR (qPCR), forward and reverse primers and appropriate probes were designed for rs1111875 from a previous Russian study (30).

Forward primer GCACCATACATCATCATAA, and reverse primer GACCAGACTAATTCAATA AATG. The fluorescent probe A HEX-AGTCATTT CCTCTAGACGTCTGAACC-BHQ-1 and probe G FAM-AGTCATTTCCTCTGGACGTCTGAA CC-BHQ-1, the annealing temperature is 56 °C.

Real-time PCR allelic discrimination assay

Detection of fluorescent signals from each probe during DNA amplification was performed using the AriaMx Real-time PCR instrument (Agilent, USA).

Fluorescein Amidite (FAM) was used to detect the G allele, and Hexachloro-Fluorescein (HEX) was used to detect the A allele. Both probes used Black Hole Quencher 1 (BHQ-1) as the fluorescence quencher. Ingredients of the reaction mixture for each sample in qPCR included: 4 µL of 5x HOT FIREPol® Probe qPCR Mix Plus (Solis BioDyne, Estonia), 1 µL of each 10 µM primer and probe (Macrogen, South Korea), 10 µL of nuclease-free water (Promega, USA) and 2 µL of template DNA. The thermal profile consists of one cycle of pre-denaturation at 95 °C for 12 minutes, fifty cycles of 30 seconds of denaturation at 95 °C, and 90 seconds of annealing/extension at 56 °C for rs1111875. Amplification and allelic discrimination plots are shown at the end of the run.

Statistical analysis

A Chi-square test (X^2) was used to determine whether one categorical variable was associated with another independent variable. The chi-square *p*-value was calculated for rs1111875, and those with a *p*-value<0.05 were significant. To decide the effect of different medical variables including FBG (mg/dL), HbA1c (%), TC (mg/dL), HDL-C (mg/dL), LDL-C (mg/dL), TG (mg/dL), VLDL-C (mg/dL), Fasting C-peptide (ng/ml) or HOMA score, ANOVA *F*-test and Kruskal-Wallis *H*-test was used. To determine the risk associated with genotypes, OR with 95% confidence Intervals (CI) were calculated using the SNPstats web tool (https://www.snpstats.net/).

Results

Demographic characteristics and clinical parameters

A total of 200 participants (one hundred patients with T2D and one hundred control) with matched ages (30-70 years) and sex (50 male and 50 female) were included in the study. The average calculated age was 52.5 ± 7.7 years and 51.3 ± 7.5 years for patients with T2D and control, respectively. Optimal glycemic control with HbA1c levels lower than 7% was observed in 26% of patients with T2D, while poor glycemic control occurs among 74% of patients with T2D. Most patients with T2D (62%) were 50-66 years old, and (53%) had been diagnosed in less than ten years. Eighty-one percent of patients with T2D have a familial history of diabetes. Heart disease was diagnosed in 40% of patients, and 67% of patients were smokers. Several patients with T2D have been taking medications to control lipid profile (20%) or blood pressure (40%) levels. The demographic characteristics of both groups are summarized in (Table 1). High association and correlation were found between the risk of T2D and having a family history of T2D (p<0.001, r =2.0), having heart disease (p<0.001, r=3.0), or being a smoker (p<0.001, r=1.7). The association and correlation between these three demographic characteristics and T2D are presented in (Table 2).

Patients with T2D had significantly higher BMI, WC, and SBP (p<0.05), while DBP was nearly comparable (p=0.312) to the control. FBS, HbA1c, TG, VLDL-C, C-peptide, and insulin resistance parameters significantly increased in patients with T2D compared to the control group (p<0.05). On the other hand, TC, HDL-C, LDL-C, and β-cells function and sensitivity were markedly higher in control subjects compared to patients with T2D (p<0.05). The clinical characteristics in terms of mean ± standard deviation for both groups are found in (Table 3).

Real-time PCR genotyping

The qPCR amplification plots, and allelic discrimination assay results are shown in (Figure 3 and Figure 4, respectively).

Associations of HHEX (rs1111875) gene polymorphism with T2D

The rs1111875 A allele was present in 41% of patients with T2D and showed significantly increased T2D risk (OR= 1.98, 95% CI= 1.30-3.02, *p*=0.002). In the genotype frequency of rs1111875, both AG (OR=3.00, 95% CI= 1.64-5.50, p<0.001) and AA (OR= 4.00, 95% CI= 1.10-14.52, *p*<0.035) showed an increased risk of T2D three to four times, respectively (Table 4).

After adjusting by age, sex, BMI, WC, family history of T2D, heart disease, hypertension, and smoking,

Table 1. Demographic characteristics of patients with T2D and control.

Characteristics	Control (n=100) n (%)	Patients with T2D (n=100) n (%)
Gender		
Male	50%	50%
Female	50%	50%
Age		
33-49	40%	38%
50-66	60%	62%
Socioeconomic status (SES)	7%	4%
Single	90%	88%
Married	1%	4%
Divorced	2%	4%
Widowed		
Glycemic control (HbA1c %)		
Optimal control (<7%)	100%	26%
Poor control (≥7%)	0%	74%
Duration of T2D (years)		
<10	-	53%
10-20	-	33%
≥20	-	14%
Having a family history of T2D		
Yes	41%	81%
No	59%	19%
Having heart disease		
Yes	4%	40%
No	96%	60%
Smoking		
Yes	39%	67%
No	61%	33%
Having lipid control medication		
Yes	3%	20%
No	97%	80%
Having hypertension control medication		
Yes	3%	40%
No	97%	60%

Abbreviations: T2D: Type 2 diabetes; %: percentage, n: number of samples

the SNPStats website (https://www.snpstats.net/) was used to analyze the difference of genotypes between patients and control in codominant, dominant, recessive, and overdominant models within rs1111875

Table 2. Association and correlation between demographic characteristics and T2D.

Demographic Characteristics T2D – Control (Ref)		*p*Ass - OR (95% CI) [a]	r(*p*cor) [b]
Having a family history of T2D	**No**	Ref	Ref
	Yes	**<0.001*** - 6.14 (3.2-11.6)	2.0 (**<0.001***)
Having heart disease	**No**	Ref	Ref
	Yes	**<0.001*** - 16.0 (5.5-47.0)	3.0 (**<0.001***)
Smoking	**No**	Ref	Ref
	Yes	**<0.001*** - 3.18 (1.8-5.7)	1.7 (**<0.001***)

Abbreviations: OR: Odd Ratio, *p*Ass: *p*-value for the association, r: correlation coefficient, *p*cor: *p*-value for the correlation coefficient, ([a]) Chi-square, and ([b]) binary logistic regression were used. **p*-value<0.05 was considered as significant

(Table 5). The codominant, dominant, and overdominant models for rs1111875 SNP showed a statistical difference in genotypes between the control and patients with T2D with a *p*-value equal to 0.01, 0.003, and 0.012, respectively. The dominant model indicates that having at least one A allele will increase T2D risk three times (OR =3.82, 95% CI= 1.55-9.41), which was also confirmed in the overdominant model as the AG genotype increases T2D risk compared to GG and AA genotypes (OR=2.96, 95% CI= 1.24-7.07).

Associations of HHEX (rs1111875) genotypes with the investigated parameters

There was no significant association between rs1111875 SNP and any clinical characteristics (BMI, WC, FBG, HbA1c, TC, TG, HDL-C, LDL-C, VLDL-C, C-peptide, insulin resistance, β-cell function, and insulin sensitivity) among patients with T2D or control ($p>0.05$) (Table 6).

Discussion

This study showed a significant difference between patients with T2D and the control in BMI and WC patients with T2D ($p<0.001$), which is consistent with the Jordanian study that showed obesity increases

Table 3. Clinical parameters and Biochemical Characteristics of patients with T2D and control.

Clinical parameters and Biochemical characteristics		Control (n=100) (Mean ± SD)	Patients with T2D (n=100) (Mean ± SD)	*p*-value
BMI (Kg/m²)		27.4 ± 4.9	32.0 ± 6.5	**<0.001**[*b]
WC (cm)	**Males**	96.1 ± 12.1	106.7 ± 16.9	**<0.001**[*b]
	Females	85.2 ± 12.9	97.7 ± 19.8	**<0.001**[*b]
SBP (mmHg)		131.4 ± 18.2	141.3 ± 24.3	**0.001**[*a]
DBP (mmHg)		82.1 ±11.4	84.4 ± 12.9	0.312 [b]
FBG (mg/dL)		92.3 ± 10.3	189.7 ± 89.7	**<0.001**[*b]
HbA1c (%)		5.29 ± 0.2	8.72 ± 2.2	**<0.001**[*b]
TC (mg/dL)		208.8 ± 43.4	190.5 ± 48.7	**0.003**[*b]
TG (mg/dL)		146.2 ± 93.2	197.2 ± 146.2	**0.006**[*b]
HDL-C (mg/dL)		47.4 ±10.9	44.1 ± 14.1	**0.024**[*b]
LDL-C (mg/dL)		134.9 ± 39.4	114.0 ± 43.0	**<0.001**[*b]
VLDL-C (mg/dL)		26.5 ± 11.6	31.0 ± 16.5	**0.002**[*b]
C-peptide (ng/dL)		2.42 ± 0.8	4.08 ± 2.9	**<0.001**[*b]
HOMA2-IR		1.77 ± 0.6	3.99 ± 3.1	**<0.001**[*b]
HOMA2-%B		135.5 ± 39.3	87.9 ± 75.1	**<0.001**[*b]
HOMA2-%S		63.1 ± 20.8	45.0 ± 50.3	**<0.001**[*b]

Clinical parameters for patients with T2D and control are presented as mean ± standard deviation (SD). *Abbreviations:* WC: Waist Circumference; SBP: Systolic Blood Pressure; DBP: Diastolic Blood Pressure; FBG: Fasting Blood Glucose: TC: Total Cholesterol; TG: Triglycerides; HDL-C: High-Density Lipoprotein Cholesterol; LDL-C: Low-Density Lipoprotein Cholesterol; VLDL-C: Very Low-Density Lipoprotein Cholesterol; C-peptide: Connecting peptide; HOMA2-IR: Homeostatic Model Assessment of Insulin resistance; HOMA2-%B: Homeostatic Model Assessment of β-cell function, HOMA2-%S: Homeostatic Model Assessment of Insulin sensitivity. *p*-value obtained by (a) Independent samples *t*-test or (b) Mann Whitney *U*-test. *p-value<0.05 was considered as significant.

the risk of T2D (31) as a result of obesity, adipose tissue produces more free fatty acid (FFA), glycerol, hormones, and pro-inflammatory cytokines that might lead to insulin resistance (32).

Significantly increased SBP levels were observed among patients with T2D compared to the control group (p= 0.001). Comparing the mean of FBS and HbA1c levels among patients with T2D and the control group, a highly significant association was observed (p<0.001), primarily due to the inclusion criteria according to the ADA diagnostic guidelines for both groups. According to Alafifi, a significant difference was found in FBG levels between patients with T2D and controls (p<0.001) among the Saudi population (33), while Al Ali *et al*. found a significant difference in HbA1c levels between patients with T2D and controls (p<0.001) among Emirati (34). HbA1c of <7% was determined as the optimal target for glycemic control among patients with T2D, and its concentrations could predict CVD risk (35). In this study, 74% of patients with T2D had poor glycemic control with HbA1c levels ≥7%, and 35% of them had heart diseases. A higher CVD is predicted among patients with T2D in the Jordanian population based on this result. High TG and low HDL-C levels are indicators for lipid metabolism disorders within T2D (36), were observed in this study as patients with T2D had a significant increase in TG levels (p<0.002) and decreased HDL-C levels (p<0.024) compared to the control group. Insulin resistance influences the production of FFA and VLDL-C from adipose tissue and liver cells, respectively (37), which explains the significant difference in VLDL-C levels between T2D and control (p=0.002). TC and LDL-C levels increase in

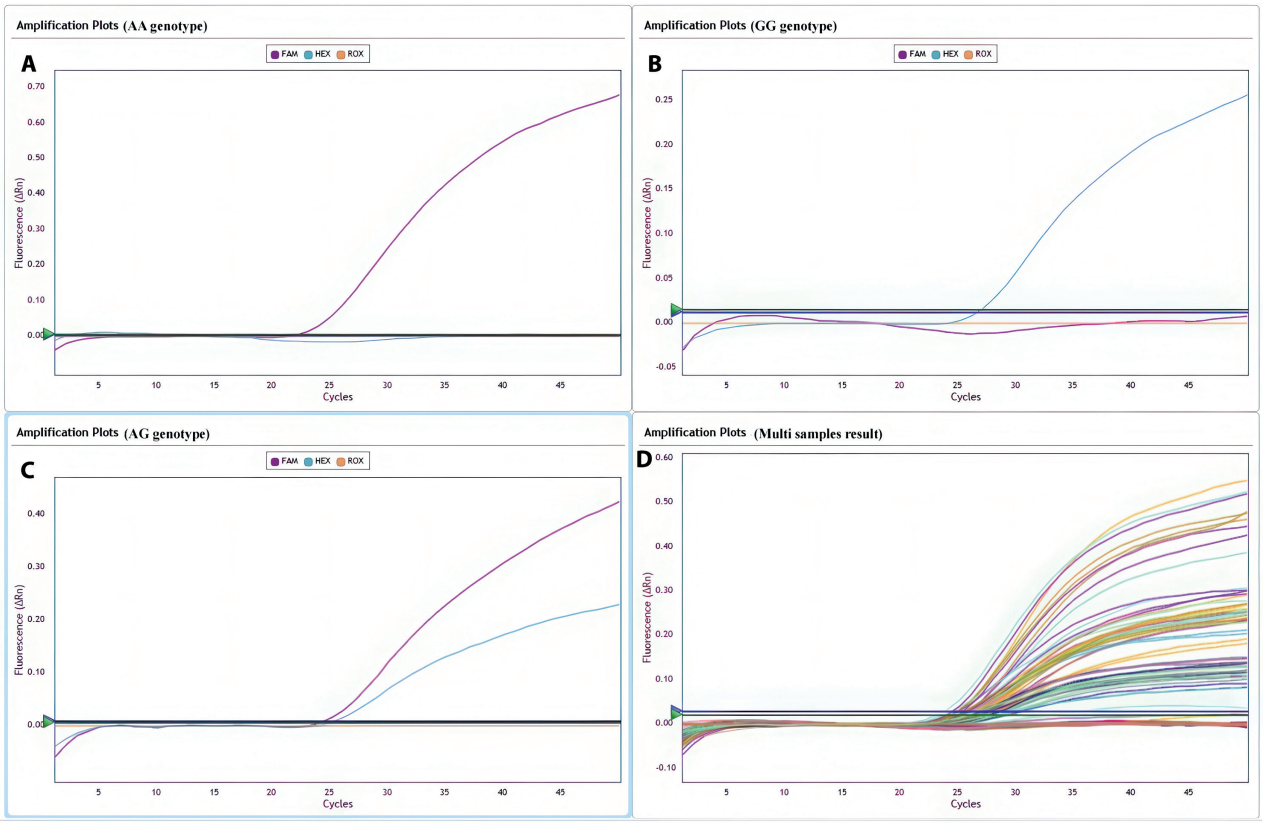

Figure 3. qPCR amplification plots. FAM dye was used with the G allele and HEX dye with the A allele, (a) the AA genotype only showed a curve for HEX, (b) the GG genotype showed only a FAM curve, (c) the AG genotype showed curves for both FAM and HEX, while (d) represents multi-samples result.

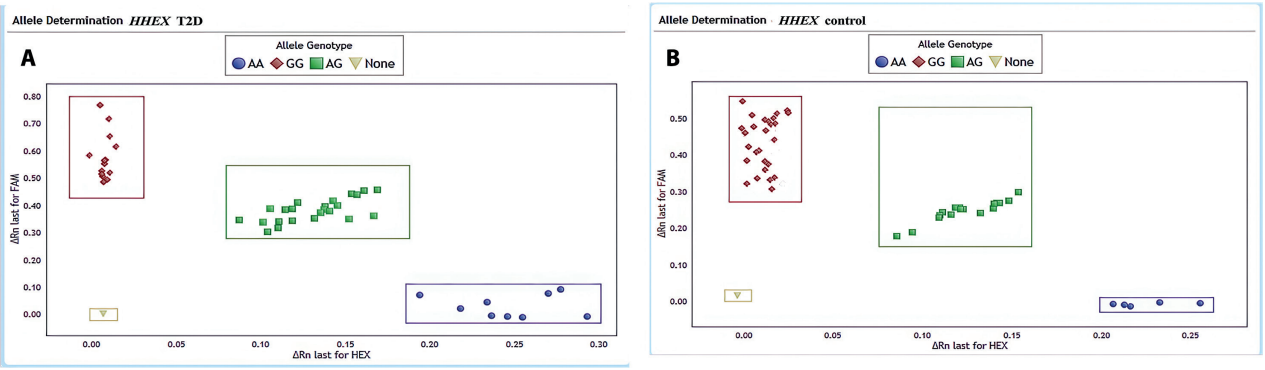

Figure 4. qPCR allelic discrimination assay results. The y-axis represents the delta normalized reporter (ΔRn) for FAM, and the x-axis represents the ΔRn for HEX, (a) *HHEX* for the T2D group, and (b) *HHEX* for the control group. AA genotype (blue circles), AG genotype (green squares), GG genotype (red diamonds), negative control (yellowish triangle), positive control for rs1111875 (AA) within blue circles.

Table 4. Allele, genotype, and Carriage rate frequencies for control and patients with rs1111875 SNPs.

Gene / SNP ID	Allele/Genotype		Control (n=100) n (%)	Patients with T2D (n=100) n (%)	X^2	*p*-value	OR (95% CI)
HHEX **rs1111875**	Allele frequency	G	148 (74%)	118 (59%)	10.1	**0.002***	1.00
		A	52 (26%)	82 (41%)			1.98 (1.30-3.02)
	Genotype frequency	GG	52 (52%)	26 (26%)	14.40	1	1
		AG	44 (44%)	66 (66%)		**<0.001***	3.00 (1.64-5.50)
		AA	4 (4%)	8 (8%)		**0.035***	4.00 (1.10-14.52)
	Carriage rate	G	96 (96%)	92 (92%)	4.09	**0.04***	1.00
		A	48 (48%)	74 (74%)			1.61 (1.01-2.55)

Abbreviations: n: number of samples, %: percentage, T2D: type 2 diabetes, X^2*:* Chi-square, OR: Odd Ratio. **p*-value<0.05 was considered statistically significant.

Table 5. Genetic model association analysis between patients with T2D and control within rs1111875.

Gene/ SNP ID	Genotype	Control (n=100) n (%)	Patients with T2D (n=100) n (%)	*p*-value	OR (95% CI)
HHEX **rs1111875**	**Codominant model**				
	GG	52 (52%)	26 (26%)	**0.01***	1
	AG	44 (44%)	66 (66%)		3.69 (1.47-9.28)
	AA	4 (4%)	8 (8%)		5.08 (0.74-34.75)
	Dominant model				
	GG	52 (52%)	26 (26%)	**0.003***	1
	AG + AA	48 (48%)	74 (74%)		3.82 (4.55-9.41)
	Recessive model				
	GG + AG	96 (96%)	92 (92%)	0.330	1
	AA	4 (4%)	8 (8%)		2.47 (0.38-16.08)
	Overdominant model				
	GG + AA	56 (56%)	34 (34%)	**0.012***	1
	AG	44 (44%)	66 (66%)		2.96 (1.24-7.07)
	Log-additive				
	---	---	---	**0.004***	2.96 (1.39-6.31)

It was adjusted by age, sec, SES, BMI, WC, family history of T2D, heart disease, hypertension, and smoking.
Abbreviations: n: number of samples, T2D: type 2 diabetes, CI: Confidence Intervals.
**p*-value<0.05 was considered statistically significant.

patients with T2D due to insulin resistance (38). Our study found that C-peptide and insulin resistance were significantly higher in patients with T2D compared to the control group (p<0.001). Similar results were reported by Ibrahim *et al*., as C-peptide (p=0.006) and insulin resistance (p=0.011) were associated with patients with T2D among Egyptians (39), probably due to the accumulation of lipid metabolites within the

Table 6. Association between rs1111875 SNP and the clinical characteristics of patients with T2D and control.

HHEX rs1111875		Control n=100			Patients with T2D n=100		
Clinical characteristics	**Genotype**	**n**	**Mean ± SD or Median (IQR)**	**p-value**	**n**	**Mean ± SD or Median (IQR)**	***p*-value**
BMI	**GG**	52	27.0 ± 4.8	0.48[a]	26	32.0 ±6.4	0.36[a]
	AG	44	28.0 ± 5.1		66	32.4 ±6.5	
	AA	4	26.0 ± 3.4		8	28.9 ±6.5	
WC	**GG**	52	87.6 ± 14.6	0.05[a]	26	103.0 (31.0)	0.56[b]
	AG	44	94.3 ± 11.4		66	105.0 (20.0)	
	AA	4	89.0 ± 16.9		8	96.0 (24.0)	
FBG	**GG**	52	91.9 ± 10.5	0.92[a]	26	162.2 (117.2)	0.46[b]
	AG	44	92.6 ± 10.1		66	160.7 (115.5)	
	AA	4	93.6 ± 10.6		8	132.1 (23.6)	
HbA1c	**GG**	52	5.3 (0.3)	0.72[b]	26	8.1 (3.1)	0.17[b]
	AG	44	5.3 (0.3)		66	8.0 (3.0)	
	AA	4	5.4 (0.2)		8	7.0 (1.9)	
TC	**GG**	52	118.9 (105.2)	0.06[a]	26	204.1 (67.0)	0.51[b]
	AG	44	220.0 ± 44.7		66	187.7 (52.7)	
	AA	4	190.4 ± 50.9		8	195.7 (106.5)	
TG	**GG**	52	102.3 (88.0)	0.57[b]	26	158.6 (138.9)	0.93[b]
	AG	44	120.5 (76.1)		66	159.0 (112.5)	
	AA	4	206.0 (353.9)		8	125.9 (553.3)	
HDL-C	**GG**	52	46.0 (15.7)	0.17[b]	26	39.5 (18.5)	0.47[b]
	AG	44	42.9 (12.8)		66	42.9 (18.9)	
	AA	4	40.8 (24.8)		8	40.8 (37.9)	
LDL-C	**GG**	52	138.0 (48.9)	0.73[b]	26	112.5 (50.9)	0.99[b]
	AG	44	131.0 (52.2)		66	115.4 (58.1)	
	AA	4	121.1 (49.9)		8	104.0 (103.5)	
VLDL-C	**GG**	52	20.6 (14.7)	0.06[b]	26	26.0 (30.1)	0.86[b]
	AG	44	24.2 (13.5)		66	28.0 (14.5)	
	AA	4	33.4 (42.5)		8	23.2 (60.0)	
C-peptide	**GG**	52	2.4 ± 0.9	0.48 [a]	26	3.5 (3.3)	0.93[b]
	AG	44	2.4 ± 0.6		66	3.3 (1.9)	
	AA	4	2.9 ± 1.3		8	3.3 (4.1)	
HOMA2-IR	**GG**	52	1.6 (0.8)	0.84[b]	26	3.5 (3.0)	0.79[b]
	AG	44	1.6 (0.7)		66	3.1 (2.5)	
	AA	4	2.0 (1.9)		8	2.8 (3.4)	
HOMA2-%B	**GG**	52	130.3 (41.6)	0.79[b]	26	72.4 (117.0)	0.91[b]
	AG	44	125.9 (35.9)		66	72.6 (87.3)	
	AA	4	128.7 (59.4)		8	71.2 (54.2)	

HHEX rs1111875		Control n=100			Patients with T2D n=100		
Clinical characteristics	**Genotype**	**n**	**Mean ± SD or Median (IQR)**	**p-value**	**n**	**Mean ± SD or Median (IQR)**	***p*-value**
HOMA2-%S	**GG**	52	63.4 (29.5)	0.84[b]	26	28.9 (29.5)	0.79 [b]
	AG	44	63.6 (24.2)		66	32.2 (24.4)	
	AA	4	55.8 (46.4)		8	37.3 (41.2)	

Abbreviations: IQR: Interquartile Range, BMI: Body Mass Index; WC: Waist Circumference; FBG: Fasting Blood Glucose; HbA1c: Glycosylated hemoglobin; TC: Total Cholesterol; TG: Triglycerides; HDL-C: High-Density Lipoprotein Cholesterol; LDL-C: Low-Density Lipoprotein Cholesterol; VLDL-C: Very Low-Density Lipoprotein Cholesterol; C-peptide: Connecting peptide; HOMA2-IR: Homeostatic Model Assessment of Insulin resistance; HOMA2-%B: Homeostatic Model Assessment of β-cell function, HOMA2-%S: Homeostatic Model Assessment of Insulin sensitivity. The *p*-value was obtained from ([a]) the analysis of variance (ANOVA) test or ([b]) the Kruskal Wallis test to determine the association between genotype and clinical characteristics.
*p-value<0.05 was considered statistically significant

liver and skeletal muscle causes impaired insulin sensitivity (37). Insulin sensitivity and β-cell function were significantly lower in patients with T2D compared to the control group (p<0.001). Insulin sensitivity is influenced by genetic, lifestyle, and environmental factors, which contribute to the development of insulin resistance, a key feature of T2D (40). Additionally, the β-cell function is impaired in T2D due to chronic exposure to high glucose levels and FFAs, which leads to insufficient insulin secretion (41). *HHEX* gene encodes a transcription factor involved in the Wnt signaling pathway, an essential pathway for cell growth and development, including the pancreas ((11, 14). *HHEX* mutation reduces the β-cells mass and secretory capacity, increasing T2DM probability (42). Several studies showed that *HHEX* rs1111875 gene polymorphism is associated with T2D within different populations, including Japanese (43-45), Chinese (46, 47), Korean (14), Indian (48), Dutch (49), Iranian (50), and Tunisian (21). However, genetic variants have varying effects on T2D risk among different ethnic groups. Chauhan *et al.* reported that there was a significant association between rs1111875 SNP of the *HHEX* gene among Indians (48). In contrast, Kommoju *et al.* reported that there was no significant association within another Indian group (22). Our study found that the rs1111875 A allele is associated with increased T2D risk (p=0.002). A similar finding was found in the Dutch population (p=0.027) (49). Tunisian population found that increased T2D risk is associated with the G allele (p=1.38×10^{-4}) (21). In contrast, the Bangladesh population showed an increased risk of T2D with those who carry the C allele (p<0.001) (Aka *et al.*, 2021). It is widely recognized that genetic predispositions to certain diseases vary significantly among different populations due to evolutionary, environmental, and demographic factors (51). To the best of our knowledge, this is the first study in Jordan that investigated the association between *HHEX* (rs1111875) gene polymorphisms and the risk of T2D. However, the study findings are valuable; they must be interpreted within certain limitations. One such limitation is the small sample size. Furthermore, the study's data was from a single hospital, which introduces a potential source of bias that may not reflect the whole community. Therefore, conducting multi-center studies involving many polymorphisms may provide a more comprehensive understanding of the genetic determinants of T2D within the population.

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References

1. Galicia-Garcia U, Benito-Vicente A, Jebari S, et al. Pathophysiology of Type 2 Diabetes Mellitus. Int J Mol Sci. 2020;21(17). doi: 10.3390/ijms21176275
2. IDF. IDF Diabetes Atlas, 10th edn Brussels, Belgium: International Diabetes Federation; 2021 [10th:[Available from: https://diabetesatlas.org/atlas/tenth-edition/.
3. Awad SF, Huangfu P, Dargham SR, et al. Characterizing the type 2 diabetes mellitus epidemic in Jordan up to 2050. Sci Rep. 2020;10(1):21001. doi: 10.1038/s41598-020-77970-7
4. Rahman RS, Almomen F, Alajmi AA, et al. Predictors and Associated Risk Factors of Development of Type 2 Diabetes Mellitus. Journal of Healthcare Sciences. 2022;02(06): 100-5. doi: 10.52533/johs.2022.2603
5. Salzberg L. Risk Factors and Lifestyle Interventions. Prim Care. 2022;49(2):201-12. doi: 10.1016/j.pop.2021.11.001
6. Witka BZ, Oktaviani DJ, Marcellino M, Barliana MI, Abdulah R. Type 2 Diabetes-Associated Genetic Polymorphisms as Potential Disease Predictors. Diabetes Metab Syndr Obes. 2019;12:2689-706.d oi: 10.2147/dmso.S230061
7. Mahajan A, Go MJ, Zhang W, et al. Genome-wide trans-ancestry meta-analysis provides insight into the genetic architecture of type 2 diabetes susceptibility. Nat Genet. 2014;46(3):234-44.doi: 10.1038/ng.2897
8. Sirdah MM, Reading NS. Genetic predisposition in type 2 diabetes: A promising approach toward a personalized management of diabetes. Clin Genet. 2020;98(6):525-47. doi: 10.1111/cge.13772
9. Kharroubi AT, Darwish HM. Diabetes mellitus: The epidemic of the century. World J Diabetes. 2015;6(6):850-67. doi: 10.4239/wjd.v6.i6.850
10. Morgutti M, Demori E, Pecile V, et al. Genomic organization and chromosome mapping of the human homeobox gene HHEX. Cytogenet Cell Genet. 2001;94(1-2):30-2. doi: 10.1159/000048778
11. Galavi H, Mollashahee-Kohkan F, Saravani R, et al. HHEX gene polymorphisms and type 2 diabetes mellitus: A case-control report from Iran. J Cell Biochem. 2019;120(10): 16445-51. doi: 10.1002/jcb.28788
12. Soufi A, Jayaraman PS. PRH/Hex: an oligomeric transcription factor and multifunctional regulator of cell fate. Biochem J. 2008;412(3):399-413. doi: 10.1042/BJ20080035
13. Verma AK, Goyal Y, Bhatt D, et al. Association Between CDKAL1, HHEX, CDKN2A/2B and IGF2BP2 Gene Polymorphisms and Susceptibility to Type 2 Diabetes in Uttarakhand, India. Diabetes Metab Syndr Obes. 2021; 14:23-36. doi: 10.2147/DMSO.S284998
14. Lee YH, Kang ES, Kim SH, et al. Association between polymorphisms in SLC30A8, HHEX, CDKN2A/B, IGF2BP2, FTO, WFS1, CDKAL1, KCNQ1 and type 2 diabetes in the Korean population. J Hum Genet. 2008;53(11-12):991-8. doi: 10.1007/s10038-008-0341-8
15. Napolitano T, Silvano S, Ayachi C, et al. Wnt Pathway in Pancreatic Development and Pathophysiology. Cells. 2023;12(4):565. doi: 10.3390/cells12040565
16. Zhang J, McKenna LB, Bogue CW, Kaestner KH. The diabetes gene Hhex maintains delta-cell differentiation and islet function. Genes Dev. 2014;28(8):829-34. doi: 10.1101 /gad.235499.113
17. Jackson JT, Nutt SL, McCormack MP. The Haematopoietically-expressed homeobox transcription factor: roles in development, physiology and disease. Front Immunol. 2023;14:1197490.doi: 10.3389/fimmu.2023.1197490
18. Qian Y, Lu F, Dong M, et al. Genetic variants of IDE-KIF11-HHEX at 10q23.33 associated with type 2 diabetes risk: a fine-mapping study in Chinese population. PLoS One. 2012;7(4):e35060. doi: 10.1371/journal.pone.0035060
19. Li X, Li Y, Song B, et al. Hematopoietically-expressed homeobox gene three widely-evaluated polymorphisms and risk for diabetes: a meta-analysis. PLoS One. 2012; 7(11):e49917. doi: 10.1371/journal.pone.0049917
20. Aka TD, Saha U, Shati SA, et al. Risk of type 2 diabetes mellitus and cardiovascular complications in KCNJ11, HHEX and SLC30A8 genetic polymorphisms carriers: A case-control study. Heliyon. 2021;7(11):e08376. doi: 10.1016 /j.heliyon.2021.e08376
21. Kifagi C, Makni K, Boudawara M, et al. Association of genetic variations in TCF7L2, SLC30A8, HHEX, LOC387761, and EXT2 with Type 2 diabetes mellitus in Tunisia. Genet Test Mol Biomarkers. 2011;15(6):399-405. doi: 10.1089/gtmb.2010.0199
22. Kommoju UJ, Samy SK, Maruda J, et al. Association of CDKAL1, CDKN2A/B & HHEX gene polymorphisms with type 2 diabetes mellitus in the population of Hyderabad, India. Indian J Med Res. 2016;143(4):455-63. doi: 10.4103/0971-5916.184303
23. Pascoe L, Tura A, Patel SK, et al. Common variants of the novel type 2 diabetes genes CDKAL1 and HHEX/ IDE are associated with decreased pancreatic beta-cell function. Diabetes. 2007;56(12):3101-4. doi: 10.2337 /db07-0634

24. Banihashemi P, Aghaei Meybodi HR, Afshari M, Sarhangi N, Hasanzad M. Association analysis of HHEX gene variant with type 2 diabetes risk. International Journal of Diabetes in Developing Countries. 2020;41(1):43-7. doi: 10.1007/s13410-020-00870-z
25. Zhao Q, Xiao J, He J, et al. Cross-sectional and longitudinal replication analyses of genome-wide association loci of type 2 diabetes in Han Chinese. PLoS One. 2014;9(3):e91790. doi: 10.1371/journal.pone.0091790
26. Harris J, McGee A, Andrews F, Souza JD, Sproston K. The National Survey of People with Diabetes.
27. Mayo Clinic Staff. Blood pressure chart: What your reading means - Mayo Clinic 2022 [Available from: https://www.mayoclinic.org/diseases-conditions/high-blood-pressure/in-depth/blood-pressure/art-20050982.
28. Ambiger S, Farheen F, Jaalam K, Shivalingappa J. Correlation of LDL Cholesterol Calculated by Friedewald's, Puavilai's, Vujovic's, de Cordova's and Martin's Formulae with Directly Measured LDL Cholesterol: A Cross-sectional Study. Journal of Clinical and Diagnostic Research. 2023;17(7):BC10-BC5. doi: 10.7860/JCDR/2023/60981.18231
29. Radcliffe Department of Medicine. HOMA Calculator 2017 [Available from: https://www.rdm.ox.ac.uk/about/our-clinical-facilities-and-mrc-units/DTU/software/homa.
30. Khodyrev DS, Nikitin AG, Brovkin AN, et al. The analysis of association between type 2 diabetes and polymorphic markers in the CDKAL1 gene and in the HHEX/IDE locus. Russian Journal of Genetics. 2016;52(11):1192-9. doi: 10.1134/s1022795416110065
31. Alkhalidy H, Orabi A, Alnaser K, et al. Obesity Measures as Predictors of Type 2 Diabetes and Cardiovascular Diseases among the Jordanian Population: A Cross-Sectional Study. International Journal of Environmental Research and Public Health. 2021;18(22):12187
32. Wondmkun YT. Obesity, Insulin Resistance, and Type 2 Diabetes: Associations and Therapeutic Implications. Diabetes Metab Syndr Obes. 2020;13:3611-6. doi: 10.2147/dmso.S275898
33. Alfaifi M. Contribution of genetic variant identified in HHEX gene in the overweight Saudi patients confirmed with type 2 diabetes mellitus. Saudi J Biol Sci. 2022;29(2):804-8. doi: 10.1016/j.sjbs.2021.10.028
34. Al Ali M, El hajj Chehadeh S, Osman W, et al. Investigating the association of rs7903146 of TCF7L2 gene, rs5219 of KCNJ11 gene, rs10946398 of CDKAL1 gene, and rs9939609 of FTO gene with type 2 diabetes mellitus in Emirati population. Meta Gene. 2019;21. doi: 10.1016/j.mgene.2019.100600
35. Khaw KT, Wareham N. Glycated hemoglobin as a marker of cardiovascular risk. Curr Opin Lipidol. 2006;17(6):637-43. doi: 10.1097/MOL.0b013e3280106b95
36. He L, Zheng W, Li Z, Kong W, Zeng T. Association of four lipid-derived indicators with the risk of developing type 2 diabetes: a Chinese population-based cohort study. Lipids Health Dis. 2023;22(1):24. doi: 10.1186/s12944-023-01790-7
37. Erion DM, Park HJ, Lee HY. The role of lipids in the pathogenesis and treatment of type 2 diabetes and associated co-morbidities. BMB Rep. 2016;49(3):139-48. doi: 10.5483/bmbrep.2016.49.3.268
38. Mooradian AD. Dyslipidemia in type 2 diabetes mellitus. Nat Clin Pract Endocrinol Metab. 2009;5(3):150-9. doi: 10.1038/ncpendmet1066
39. Ibrahim I, Khoreba A, Al Adl H, Talkhan H, Abdelhafeez G. Assessment of serum c-peptide level as a risk factor for atherosclerosis and myocardial dysfunction in Egyptian patients with type 2 DM. Al-Azhar International Medical Journal. 2022;0(0):0-. doi: 10.21608/aimj.2022.119805.1828
40. Latini G, Marcovecchio ML, Del Vecchio A, et al. Influence of environment on insulin sensitivity. Environ Int. 2009;35(6):987-93. doi: 10.1016/j.envint.2009.03.008
41. Khin PP, Lee JH, Jun H-S. Pancreatic Beta-cell Dysfunction in Type 2 Diabetes. European Journal of Inflammation. 2023;21:1721727X231154152. doi: 10.1177/1721727x231154152
42. Tanaka H, Yamamoto T, Ban T, et al. Hex stimulates the hepatocyte nuclear factor 1alpha-mediated activation of transcription. Arch Biochem Biophys. 2005;442(1):117-24. doi: 10.1016/j.abb.2005.08.001
43. Omori S, Tanaka Y, Takahashi A, et al. Association of CDKAL1, IGF2BP2, CDKN2A/B, HHEX, SLC30A8, and KCNJ11 with susceptibility to type 2 diabetes in a Japanese population. Diabetes. 2008;57(3):791-5. doi: 10.2337/db07-0979
44. Horikawa Y, Miyake K, Yasuda K, et al. Replication of genome-wide association studies of type 2 diabetes susceptibility in Japan. J Clin Endocrinol Metab. 2008;93(8):3136-41. doi: 10.1210/jc.2008-0452
45. Furukawa Y, Shimada T, Furuta H, et al. Polymorphisms in the IDE-KIF11-HHEX gene locus are reproducibly associated with type 2 diabetes in a Japanese population. J Clin Endocrinol Metab. 2008;93(1):310-4. doi: 10.1210/jc.2007-1029
46. Li C, Shen K, Yang M, et al. Association Between Single Nucleotide Polymorphisms in CDKAL1 and HHEX and Type 2 Diabetes in Chinese Population. Diabetes Metab Syndr Obes. 2020;13:5113-23. doi: 10.2147/DMSO.S288587
47. Lin Y, Li P, Cai L, et al. Association study of genetic variants in eight genes/loci with type 2 diabetes in a Han Chinese population. BMC Med Genet. 2010;11(1):97. doi: 10.1186/1471-2350-11-97
48. Chauhan G, Spurgeon CJ, Tabassum R, et al. Impact of common variants of PPARG, KCNJ11, TCF7L2, SLC30A8, HHEX, CDKN2A, IGF2BP2, and CDKAL1 on the risk of type 2 diabetes in 5,164 Indians. Diabetes. 2010;59(8):2068-74. doi: 10.2337/db09-1386
49. van Vliet-Ostaptchouk JV, Onland-Moret NC, van Haeften TW, et al. HHEX gene polymorphisms are associated with type 2 diabetes in the Dutch Breda cohort. Eur J Hum Genet. 2008;16(5):652-6. doi: 10.1038/sj.ejhg.5202008

50. Al-Mansoori L, Al-Jaber H, Prince MS, Elrayess MA. Role of Inflammatory Cytokines, Growth Factors and Adipokines in Adipogenesis and Insulin Resistance. Inflammation. 2022;45(1):31-44. doi: 10.1007/s10753-021-01559-z
51. Kim MS, Patel KP, Teng AK, Berens AJ, Lachance J. Genetic disease risks can be misestimated across global populations. Genome Biol. 2018;19(1):179. doi: 10.1186/s13059-018-1561-7

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