Obesity-Related Type 2 Diabetes Mellitus and HHEX Gene rs1111875 C/T Polymorphisms in Iraqi Population.

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Abstract

type 2 diabetes mellitus(T2DM) is a complex metabolic disease. Diabetes mellitus has quadrupled globally in the last three decades. Many studies have shown how genetic diversity contributes to the development and progression of T2DM. The hematopoietic expressed homeobox (HHEX) gene is hypothesized to be linked to the risk of T2DM. The aim of this study was to look at the association of the HHEX gene polymorphism (rs1111875 C/T) with T2DM in the Iraqi population and see if obese individuals were affected more than other patients. Methods: 100 T2DM obese and 100 controls obese. The RFLP -PCR assay Technique was used to genotype HHEX rs1111875 C/T, making use of newly-designed primers. Results: The study's parameters demonstrate no significant difference between T2DM obese and obese control groups. The study concluded that there is no significant difference in genotypes Codominant, Dominant, Recessive, and Additive genotype between groups for HHEX rs1111875 C/T. and Chol, TG, and VLDL were significant between CC and CT, age was borderline. There is a negative relationship between (BMI and CHO and TG) and (insulin and HOMA-IR and LDL, VLDL). In conclusion, according to the findings of this study, the rs1111875 C/T polymorphism in the HHEX gene revealed no significant difference in genotypes in any of the codominant, dominant, recessive, or additive models.

Key words: HHEX gene, obese, type 2 diabetes.

Introduction

An international health issue is diabetes[1], A metabolic condition called DM causes excessive blood sugar levels[2]. can arise from -cell malfunction, insulin resistance, or both in the context of absolute or relative insulin deprivation[3]. The most prevalent kind of diabetes is T2DM[4]. Insulin resistance and partial insulin insufficiency are traits of T2D[5]. Over 90% of

diabetes cases worldwide are caused by T2DM. And current forecasts indicate that by 2045, this number will increase to 629 million[6]. Over the previous 50 years, the prevalence of obesity has increased globally [7] Obesity is a significant risk factor for T2D, and more than two-thirds of individuals are obese at the time of diagnosis [8]. More than 90% of people with T2DM also have obesity, and more than 20% of people with obesity also have T2DM[9]. A vast number of cross-sectional and longitudinal studies show that body mass index (BMI) is associated to the risk of T2DM [10]. The pathophysiology of T2DM involves both environmental and genetic variables. Most of the implicated genes contribute to B-cell function. The vulnerability to T2DM may also be influenced by genetic variants that affect crucial proteins involved in insulin secretion and glucose metabolism. Linkage analysis, candidate gene approachesand genome-wide association studies(GWASs)have identified a number of genes that enhance the risk of T2DM [11]. HHEX are essential for the sensitivity and secretion of insulin[12]. The HHEX is also called HEX, PRHX, and PRH [15]. The HHEX gene has 4 exons and is found on chromosome 10q[13] [14]. The gene's generated protein acts as a transcription factor in the leading pathway (known as Wnt signaling) and is crucial for the initial assessment of the ventral pancreas and liver [14].

In addition, lower insulin resistance and secretion as well as decreased hepatic insulin breakdown are important and main mechanisms in the pathophysiology of T2D, and HHEX replicated T2D susceptibility loci have been linked to decreased insulin sensitivity or secretion. This made it a potential candidate gene for T2D etiology.[12]. There haven't been many genetic research on T2DM patients in Iraq. As a result, we decided to undertake the current study between the 7923837 G/A polymorphism in the HHEX gene and obese T2DM patients in Iraq.

Study subjects

A case study is being conducted and controlled on 200 people (100 T2DM and 100 controls). 100 patients 49 obese divided to (33 females and 16 males), were chosen from those who attended the Diabetes Center in Al-Sadr Teaching Hospital, Iraq, while 100 control 46 obese divided to (27 females and 19 males), were chosen from the family, friends and medical staff who came to the hospital for the examination. Fasting blood glucose, triglycerides, LDL, HDL, VLDL and insulin concentrations were measured. HOMO-IR was calculated. The

genotyping of the HHEX gene SNP (rs1111875) were performed by REFLP-PCR technique.

Extraction and genotyping of DNA

To extract DNA from frozen blood samples, a G-spinTM Total DNA Extraction Mini Kit was used: "200 µl of whole blood were mixed with 20 µl of proteinase k, then the mixture incubated at 56° C for 10 minutes, then the manufacturer's protocol was followed."

HHEX rs1111875 C/T

The RFLP-PCR technique was utilized to genotype rs1111875C/T, with freshly generated primers particularly built for this study in the UGene laboratory, as shown in Table 1.

This study's primers

All of the primers utilized in this study are novel and were created expressly for our study, and the internet server Primer3 was used for this purpose with some modifications.

Table. 1 the primer sequence for PCR amplification of HHEX gene polymorphism rs1111875 C/T.

Target gene		Sequence (5'-3')	Ta (°C)	Product size	Accession number	Designer
HHEX	F	CTATTTGGGATGGGCTGTGAT	56	450 bp	AL590080.25	UGene lab
	R	CCTCAGGCAAATGGTACTTAGG				

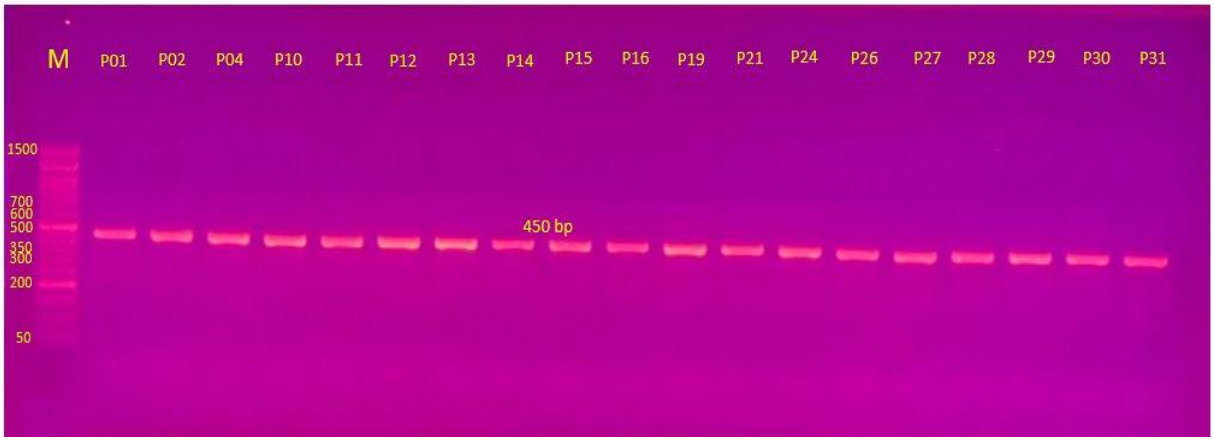

Fig.1 PCR products of the amplification of partial region of gene HHEX of Homo sapiens for detection of SNP rs1111875 C/T, the size of the PCR product is 450 bp. The gel was 1.5% and the DNA dye is RedSafe (Intron, Korea). V: 90, Time: 45 minutes. M: DNA ladder.

REFLP-PCR assay

According to the manufacturer's instructions, the AddPrep Genomic DNA Extraction Kit was use to extract the genomic DNA from 200 ml of EDTA blood. 200 DNA samples were evaluated for quantity and quality using a NanoDrop spectrophotometer. Using 50 ml of reactions and 25 ml of the GoTaq® G2 Green Master Mix kit (PROMEGA, USA), PCR was performed on the HHEX gene for the rs1111875 C/T polymorphism. Filtered water, forward and reverse primers, and the residual products were added to the reaction mixture. Separate additions of genomic DNA were made to complete the reaction. Over 35 cycles, genotyping was done. The intended band size for the PCR result was 100 bp, which was confirmed on an agarose gel dyed. The rs 1111875 C/T polymorphism's restriction site was cut using the XbaI restriction enzyme. Restriction After cleaving the restriction site with a Fragment Length Polymorphism (RFLP) assay overnight at 37 C, the products were then put through electrophoresis to be seen. For each SNP under investigation, three genotypes were anticipated.

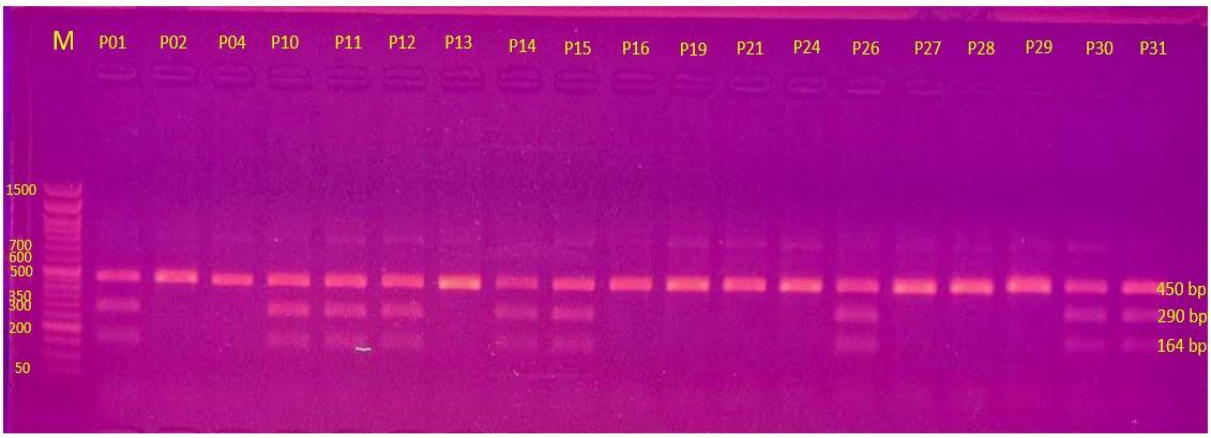

Fig.2 RFLP bands of SNP rs1111875 C/T of gene HHEX of Homo sapiens. The PCR product of these samples were digested using the restriction enzyme (XbaI). The wild type genotype (CC) would have one band (450 bp). The heterogenotype (CT) is confirmed once three bands of (450 bp, 290 bp and 164 bp) are showed in the gel. Finally, the mutant genotype (TT) is detected once the gel expressed two bands of (290 bp and 164 bp). The gel was 2% and the DNA dye is RedSafe (Intron, Korea). V: 90, Time: 45 minutes. M: DNA ladder**.**

Statistical analysis

Although the initial sample comprised 100 patients with T2DM patients and 100 healthy subjects, the analysis was conducted on 98 T2DM subjects and 99 healthy, as three samples failed to produce any findings, presumably due to denaturation of their nucleic acids caused by thawing / freezing. SPSS v.20.0 (PASW Statistics, Journal Preproof 8 SPSS Inc., Chicago, IL, USA) was used to analyze the data. All clinical and demographic data, which were presented as mean SE, were analyzed using the t-test and the Chi-square($\chi 2$) test. Using the frequency of the target gene in healthy people as the foundation, SNP-Analyser version 1.15 ga basic analysis ($p > 0.05$) was done until the Hardy-Weinberg equilibrium was reached. ANOVA was used to compare the rs1111875 polymorphism to T2DM details. The existence of genotypes was compared between healthy participants and patients using the odds ratio (OR) with a 95% confidence interval (95% CI). In the comparative analysis, a p-value of less than 0.05 was considered significant.

Results

The current study covers 200 individual's 100 patients and 100 control , consists of two groups.

G1: Control obese

G2: Patient obese

T2DM patients and controls had mean ages of (49.01000.76890) and (44.25250.85827), respectively. Obese T2DM patients had an mean BMI of (35.0289±0.69349), Obese control had an mean BMI of (34.7882±0.78126). Our findings revealed statistically significant variations in biochemical and lipid profile features for diabetes criteria between control obesity and T2DM patients obese, as illustrated in tables 2 and 3.

Table. 2 a summary of T2DM biochemical characteristics and control groups.

Groups / ***Parameters***	**Mean**	**SE**	**Compared Groups**		**Sign.**
FG G1	98.9565	1.59163	1	2	0.897
				3	0.000
				4	0.000
G2	179.8571	8.86597	3	1	0.000
				2	0.000
				4	0.927
Insulin G1	10.2065	1.42506	1	2	0.864
				3	0.006
				4	0.030
G2	16.1112	1.59474	3	1	0.006
				2	0.008
				4	0.542
HOMA-IR	2.5572	0.34424	1	2	0.902

G1					
				3	0.027
				4	0.104
G2	4.9515	1.13059	3	1	0.027
				2	0.031
				4	0.533

Table. 3 summary of the lipid profile parameters of T2DM and control groups.

Groups / *Parameters*	*Mean*	*SE*	*Compared Groups*		*Sign.*
Chol G1	162.9783	7.31879	1	2	0.287
				3	0.993
				4	0.119
G2	162.8980	6.04041	3	1	0.993
				2	0.283
				4	0.110
TG G1	142.9587	11.98370	1	2	0.134
				3	0.012
				4	0.000
G2	189.3796	12.23119	3	1	0.012
				2	0.000
				4	0.013
HDL G1	39.2565	1.30968	1	2	0.425
				3	0.006
				4	0.751
G2	33.1367	1.56705		1	0.006

			3	2	0.000
				4	0.012
LDL G1	98.6043	5.49919	1	2	0.252
				3	0.162
				4	0.350
G2	89.3449	5.32902	3	1	0.162
				2	0.776
				4	0.627
VLDL G1	28.5976	2.40047	1	2	0.066
				3	0.003
				4	0.003
G2	37.9290	2.43701	3	1	0.003
				2	0.000
				4	0.947

Table. 4 according to the HWE, SNP rs 1111875 C/T in the human gene HHEX study of the genotype of the Control subject.

Genotype of control	Observed	Expected	Difference	X^2	P value
CC Reference	44	46.0227		0.8781	0.3487
CT Heterozygote	47	42.9545			
TT Recessive	8	10.0227			
C allele%	68.18				
T allele%	31.82				

the genotype frequencies of gene HHEX SNP rs 1111875 C/T are compatible for control with hardy weinberg equilibrium regulation.

Table. 5 genotype of rs1111875 C/T polymorphism of obesity control and obese patients.

rs1111875 C/T	Control N=46	Patients N=48	OR (CI 95%) P value	Adjusted OR
Codominant				
CC	21	21	1	1
CT	20	23	1.15 (0.4908 to 2.6945) 0.7	1.18 (0.494-2.81) 0.71

TT	5	4	0.8 (0.1882 to 3.4012) 0.76	0.72 (0.165-3.17) 0.67
Dominant				
CT+TT	25	27	1.1 (.4788 to 2.4363) 0.85	1.1 (0.321-4.12) 0.84
Recessive				
CC+CT	41	44	1	1
TT	5	4	0.7 (0.1872 to 2.9688) 0.67	1.4 (0.3842-2.14) 0.62
Additive				
2CC+CT	62	65	1	
2TT+CT	30	31	0.98 (0.5351 to 1.8154) 0.96	

table represents the comparison between the obese control and obese patient groups no significant variation was observed in (codominant, dominant, and additive models respectively).

Table.6 clinical parameters of DM groups according to HHEX gene polymorphism SNP rs 1111875 C/T genotype (codominant model).

Clinical parameter	CC N=44	CT N=46	TT N=8	P value
Age(Y)	47.636±1.116	50.608±1.1363	51.250±1.332	0.05
BMI (kg/m^2)	29.549±1.030	29.008±0.935	30.166±2.596	0.701
FG (mg/dl)	187.681±12.335	172.173±11.040	175.750±24.608	0.347
Chol(mg/dl)	183.363±7.610	158.456±5.251	169.125±14.628	0.008
TG (mg/dl)	252.104±17.919	172.463±12.644	214.062±38.528	0.000
HDL (mg/dl)	36.445±1.650	35.565±1.958	34.250±3.905	0.731
LDL (mg/dl)	96.353±5.992	88.147±4.155	88.246±10.077	0.254
VLDL (mg/dl)	42.430±2.669	33.117±2.083	36.779±5.511	0.007
Insulin(µU/ml)	15.556±1.812	15.057±1.335	15.789±4.122	0.825
HOMA-IR	3.807±.893	5.512±1.203	4.908±1.756	0.252

this table displays the differences in Chol, TG, and VLDL between CC&CT, as well as the age at the border.

	BMI	*F. G*	*CHO.*	*TG*	*HDL*	*LDL*	*VLDL*	*Insulin*	*HOME-IR*
BMI	1								
F. G	0.06002	1							
CHO.	-0.08864	0.204818	1						
TG	-0.2183	0.171718	0.283588	1					
HDL	-0.26473	0.040194	0.489429	-0.17615	1				
LDL	0.143531	-0.08497	0.494821	-0.43969	0.322512	1			
VLDL	0.033088	-0.01637	-0.0098	0.276272	-0.18159	0.25249	1		
Insulin	-0.05685	0.075246	-0.07147	0.161759	-0.05546	-0.14844	0.149674	1	
HOME-IR	0.07088	0.448417	0.049639	-0.05898	-0.0115	0.088366	-0.06429	0.336699	1

Fig .3 Correlation of patient clinical indicators. the above figure shows correlation between lipid profile, FG, Insulin, and HOMA-IR. the color blue safe side, while pink color dangerous for patient.

Discussion

Diabetes develops when blood glucose levels are persistently high over the physiological limit (T2DM), which is a significant medical challenge of the twenty-first century [15]. T2DM It is primarily caused by the combination of two key factors: the inability of insulin-sensitive tissues to respond to insulin and inadequate insulin secretion by pancreatic beta-cells [16]. body mass index, fasting blood glucose (FBG),Age, and blood pressure all influence the chance of developing type 2 diabetes [17]. environmental, metabolic and Genetic factors all combine to raise the likelihood of developing type 2 diabetes (T2DM) [16]. A highly polygenic architecture of the illness has been discovered as a result of several GWAS studies to identify genetic risk variants for T2DM. More than 500 genetic loci have now been identified as being related with an increased risk of type 2 diabetes, and many of these loci show variation in allele frequency and impact size among genetic ancestries [18] [19][11].The HHEX gene has repeatedly been found by GWAS across ethnicities as a likely candidate for T2DM risk. because to the rising frequency of hyperglycemia. There has been a surge of interest in identifying HHEX genetic marker [13]**.** HHEX is essential for insulin sensitivity and secretion, as well as for cell growth and differentiation, The development of the blood cells liver, pancreas,

and heart in several species is similarly regulated by HHEX. It also maintains the terminal differentiation state of somatic cells, such as that of pancreatic delta cells, and inhibits proliferation [12] [20] [21]. The most common type of genetic variation is gene mutation, such as single nucleotide polymorphisms (SNPs) [22]. Several investigations have identified numerous SNPs and mutations within or close to the gene area. The common variant found close to the HHEX gene area that has undergone extensive examination because of its association with T2D risk is rs1111875 [12].The Insulin secretory response and HHEX/insulin-degrading enzyme (IDE) SNPs (rs1111875) were linked in several studies, indicating that the HHEX gene may raise the risk of type 2 diabetes by interfering with pancreatic cell activity [12] [24] [23].

Our result show rs 1111875 polymorphism there is no significant difference between controls obese and patients obese genotype in any of the codominant, dominant, recessive, and additive model's genotypes. Some studies did not match our study in Dutch Breda cohort[22] southeast Iranian[14] In Potsdam, Germany[23], while our current study was identical to the study conducted in Uttarakhand, India [24]. Another study conducted on the Thai population showed no association between rs 1111875 located near gene HHEX and T2DM[25].

Conclusions

In this study of HHEX gene rs1111875 C/T Polymorphisms, the genotype and allelic frequency differences between obese T2DM cases and obese controls were statistically non-significant in the codominant, dominant, recessive, and additive models.

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