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a special issue for the scientific conference held by the Department of Chemistry- College of Education for Girls/University of Kufa,under the title:
(6'th Postgraduate Students Annual Conference) (PSAC2025).
which held for Tuesday, 15/4/2025.

The role of the rs2144908 SNP of *the HNF4A* Gene in T2DM of Iraqi Patients

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Abstract

High blood glucose levels are a hallmark of type 2 diabetes mellitus (T2DM). A connection between genetic variations and the incidence of type 2 diabetes has been shown by several studies conducted in various nations, while others are unable to detect a noticeable outcome. In Iraq, diabetes has become an epidemic in the past ten years, paralleling the global rise in the prevalence of diabetes mellitus. The goal of our research was to examine the relationship between T2DM disease in Iraqi patients and the polymorphism of the *HNF4A* SNP rs2144908. A group of 100 healthy control individuals and *a* group of 100 T2DM patients participated in a case-control study. Using specially created primers for this research, the RT-PCR technique was utilized. The findings of the assessment of biochemical parameters showed a marked rise in the codominant, dominant, overdominant, and additive

genotype models between the two groups. In codominant characteristics, it indicates that for GG, GA, *and* AA, insulin, HOMA-IR, TG, and VLDL have shown values, while in dominant for GG vs. GA + AA, the insulin, HOMA-IR, cholesterol, TG, LDL, and VLDL have shown significant values. As we Know this is the first study in Iraq deal with this gene with T2DM, so the study indicated that the development of T2DM is significantly influenced by *HNF4A* rs2144908 G/A in Iraqi patients.

Key words: T2DM, *HNFA4* gene, rs2144908 SNP, Real Time PCR, RT_CR.

1.Introduction

Type 2 diabetes mellitus disease often occurs in the condition of resistance to insulin or progressive decrease of sufficient pancreatic β-cell insulin secretion[1-3] and this affects about 90–95% of cases of the disease. There are currently 537 million individuals aged 20 to 79 who suffer from diabetes; the patient number is expected to increase to 643 million by 2030, and by 2045, it will have reached over 783 million. Diabetes was the cause of at least 966 billion dollars in medical expenses, which is the first global target for diabetes mellitus and represents a 316% increase over the preceding 15 years [4-6]

The mutations in Gene are known to be the cause of hereditary disorders, polymorphisms of gene is the most common genetic variation in humans that do not always correlate with a particular one[7]. Single Nucleotide Polymorphism(SNP), which reveal the most prevalent kinds of polymorphisms in humans nucleotide is characterized by substitution of one nucleotide for another[8] Most of the genes involved in B-cell activity are genetic polymorphisms that affect insulin release and glucose metabolism[9-11] Genome Wide Association Study (GWAS), candidate gene techniques, and many genes linked to type 2 diabetes that have been found through these linkage studies [12].

In order to regulate the activity of specific genes, the HNF4A protein binds to specific DNA sequences, a process known as transcription factor action that is required for the human liver and pancreatic islets to operate normally [13] HNF4A regulates hepatic gluconeogenesis and secretes insulin critically[14] The maturity onset of diabetes in the young (MODY) is caused by mutations of this gene, according to prior understanding and linkage studies of T2DM that revealed potential peaks in the HNF4a region in many studies[15],The *HNF4A* genes located in the chromosome region between 20 q13.12 and 20 q13.13 have also been linked to type 2 diabetes susceptibility, according to earlier research.Human chromosome 20q12–q13 may contain one or more genes that predispose people to diabetes, according to linkage studies of type 2 diabetic patients[16].

There is evidence in populations in the North of America, Europe, and the East of Asia that there is a relationship between type 2 diabetes and variations in the *HNF-4A* gene[17]. It's believed that type 2 diabetes has become epidemically prevalent in Arab countries. Genetic variations are considered to have a major role in the pathogenesis of diabetes and may contribute to the disease's prevalence among Arabs because they are more likely to develop diabetes due to a unique genetic background[18] . The first systematic review with meta-analysis conducted across 22 Arab nations looked at the risk-associated gene variation with type 2 diabetes in Arab individuals did not mention *HNF4A* in many countries among 32 gene and 71 SNP [19].

However, the *HNF4A* gene was not investigated at that time, and as we know, this is the first study in Iraq about it, so the goal of our research is to determine whether rs4810424 of the *HNF4A* gene and type 2 diabetes in the Arab Iraqi population are related.

2. Materials and Methods

2.1 Design and subject

The association between T2DM and SNP rs2144908 G/A of the *HNF4A* was investigated in the case-control research study with 100 participants (40 female & 60 male) who visited the diabetic center at Al-Sader Medical City and were selected according to the World Health Organization's (WHO) 2021 diabetes mellitus diagnosis criteria. while 100 participants of healthy controls (44 female & 56 male) were chosen at randomly from the general population, including family members and coworkers who visited the hospital for the purpose of getting checked up, had the following criteria used in the selection: An HbA1c level less than 5.6%, no prior history of urine glucose or a test of standard glucose tolerance (75 g), and without a history of heart disease, increased blood pressure, increased level of cholesterol, or other associated disorders. The 200 participants were all citizens of Iraq, especially in Al-Najaf city.

2.2 The approval of the ethical committee

The study was carried out in accordance with the ethical guidelines outlined in the Helsinki Declaration. Before collecting the sample, the patient's verbal and analytical consent was obtained. The Faculty of Medicine's Ethical Committee at

Kufa University gave its approval to the study protocol. The permission form, subject data, and study protocol were reviewed and approved in compliance with document number 19 / 2 /2025, “Reference MEC-115 ".

2.3. Genotyping and DNA extraction

The peripheral venous blood was obtained from each subject, and then, along with EDTA-anticoagulant, Using the DNA Mini Kit, the frozen blood samples were extracted for DNA. (Twenty microliters of proteinase K were combined with 200 microliters of whole blood, and then for ten minutes, the mixture was incubated at 56 °C. After that, the procedure of the manufacturer was adhered).

2.4 *HNF4A* rs2144904 G/A

rs2144904 G/A was genotyped using the real-time PCR method. utilizing the newly created primers stated in the table below that were created especially for this investigation in the UGene lab.

2.5 The study's primers

Each primer utilized in our research is novel and formed especially for our work, and for this, a modified version of the internet server Primer3 was utilized.

Table 1. The *HNF4A* gene polymorphism rs2144908 G/A primer sequence for PCR amplification

Gene	Primers name	Sequences 5'-3'	PCR product	Accession number
HNF4A	F	CATTGCAAAGACACAATCAACA	156 bp	NG_009818.1
	R	CCATTTCCAGTGCATCACAT		
	Allele A	FAM-5'-CTGAGGACAGAGAGCCAG-3'-BHQ1		
	Allele G	HEX-5'-CTGAGGACAGAGGGCCAG-3'-BHQ1		

The table above show the sequences of each primers that used in this study and its PCR products and its accession number

2.6 Assay for Real-Time PCR

The standard real-time PCR was applied to all of the samples. The conserved portion of the Homo sapiens *HNF4A* gene was amplified using a single pair of particular primers. Using a commercial kit, DNA was isolated from leukocytes in the peripheral circulation. Primers with probes specific to SNPs were used to genotype the rs2144908 G/A and HNF4A polymorphisms. Two ng of the genomic DNA and the GoTaq® Probe qPCR Master Mix (Promega, USA) were carried out in 0.2 µl wells in a total volume of 25 ml. A thermal cycler (Stratagene, USA) was then used to heat the wells for about 10 min at 95°C, and about 40 cycles at 95°C for 15 s and 60°C for 1 min were then completed. The error rate for PCR is duplicates of < 1%, and the rate of success was > 95%.

2.7 Statistical analysis

Version 27.0.1.0 of IBM SPSS Statistics software was used to conduct statistical analyses [20]. All of the clinical and the demographic data, that given as mean ± SE, which analyzed by using both t-test and the test of Chi-square ($\chi 2$). Basic analysis ($p > 0.05$) was conducted using SNP-Analyzer version 1.15. Ultimately, the Hardy-Weinberg equilibrium was achieved by using of the frequency target gene in healthy individuals as the foundation. An ANOVA was conducted between the T2DM details and the rs2144908 polymorphism. With a percent of 95% confidence interval (95% CI), the odds ratio (OR) was used to compare the genotypes of patients and healthy subjects. According to the comparison study, a p-value of ≤ 0.05 was considered significant.

3. Results

There are 200 participants in the current study divided into two groups, 100 in one group are controls and 100 of in another group are patients. The mean age of control and patient groups are (46.614 ± 9.746) and (49.617±8.356) respectively. Also the BMI of healthy control and T2DM patients are (24.808 ±1.68) and (24.583± 2.38) respectively.

Table 2. Measurements of the Diagnostic parameters in healthy control and T2DM patient groups

Parameter	Control(Mean± SD)	T2DM Patients(Mean ± SD)	P-Value
FBS(mg/dl)	91.74 ± 12.4873	174.72± 57.429	< 0.001
Insulin(µU/ml)	7.034±7.861	9.606± 6.970	0.011
HOMA_IR	2.0966±1.8506	8.0732±7.0555	< 0.001

There is signficant diffrences between healthy control and patients regardence FBS and HOMA-IR by p-value less tha 0.001 and in insuline the p-value was 0.011.

There is significant diffrences between cholesterol, TG, LDL-c and VLDL-c by p-value less than 0.001 between healthy control and T2DM respectively in Table 3.

Table 3. Measurements of lipid profile in healthy control and T2DM patient groups

parameters	Control(Mean± SD)	T2DM Patients(Mean ± SD)	P-Value
Cholesterol (mg/dl)	150.84±27.83	184.1±46.686	< 0.001
TG (mg/dl)	121.1±51.8	167.14±66.197	< 0.001
HDL _c (mg/dl)	38.034±8.4564	40.16±10.601	0.137
LDL _c (mg/dl)	87.065±16.765	110.375±43.547	< 0.001
VLDL_c (mg/dl)	24.217±10.3412	33.652±13.1435	< 0.001

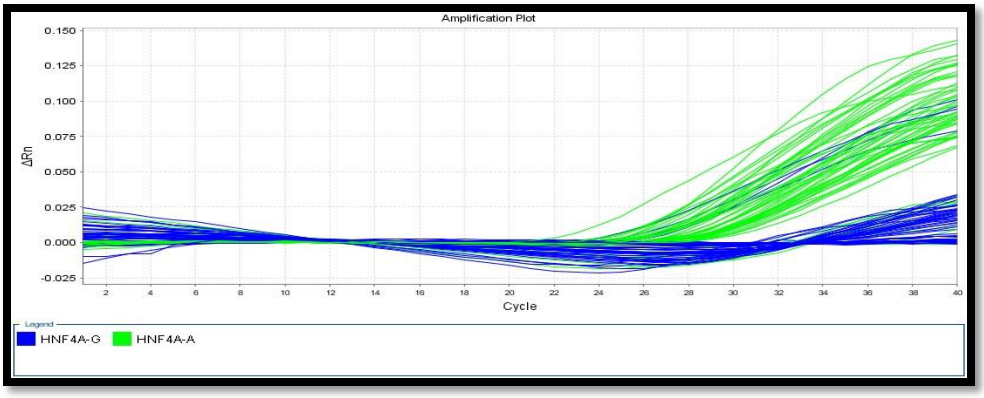

Figure 1. Real-time PCR amplification curve for the HNF4A gene's rs2144908 G/A polymorphism

The threshold is represented by the zero line, the number of heat cycles is represented by the X-axis, the fluorescence intensity is characterized by the Y-axis.while the plot diagrams are the amplification products that display the FAM probe, which has a marker reaction to the A allele, at the same time, it displays a circle marker for the HEX probe reaction to the G allele. By the building on a particular probe, the amplification curve will illustrate the fluorescent signal and different detection if an action happens in the A allele or G allele.

Table 4. *HNF4A* SNP rs2144908 G/A genotype analysis in healthy control according to HWE

Genotype of rs2144908 G/A in Control					
Genotype	Observed	Expected	Residual	X^2	p-value
GG Reference	64	65.6	-1.6	1.094	0.579
GA Heterozygote	34	30.8	3.2		
AA Recessive	2	3.6	-1.6		

The Hardy–Weinberg equilibrium in control subjects is supported by the *HNF4A* SNP rs2144908 G/A genotype frequencies.

Table 5. rs2144908 G/A polymorphism genotypes of all healthy individuals and T2DM patients

SNP rs2144908 G/A	Control NO.=100	T2DM Patients NO.=100	OR(CI 95%)	P-Value
Codominant				
GG(Reference)	64	36		
GA	34	58	3.0327(1.6842_5.4610)	0.0002
AA	2	6	5.3333(1.0226-27.8152)	0.0470
Dominant				
GA+AA	36	64	3.1605 (1.774-5.6305)	0.0001
Over Dominant				
GG+AA(Reference)	66	42		
GA	34	58	2.6807(1.5107-4.7567)	0.0008
Recessive				

GG+GA(Reference)	98	94		
AA	2	6	3.1277(0.615815.8861)	0.1691
Additive				
2(GG)+GA	162	130		
2(AA)+GA	38	70	3.2749 (1.8556-5.7797)	<0.0001

In the majority of models (codominant, dominant, over dominant, and additive), as the following table reported that there is a considerable difference between the control and T2DM groups, while in the recessive model it's not significant that shown in Table 5.

Table 6. Clinical characteristics of the T2DM groups based on the Codominant model of the HNF4A gene polymorphism SNP rs2144908

Parameters rs2144908 G/A	GG	GA	AA	P-Value
Age	50.94±8.950	48.495±7.99	46.66 ± 8.84	0.3716
BMI	23.91±1.921	25.26±2.237	23.25 ± 4.16	0.1074
FBS	176.44±73.505	175.99±48.09	139.5 ± 33.55	0.3284
Insulin	12.86±9.396	7.944±4.402	5.76 ± 1.20	0.0012
HOMA_IR	11.86±7.04	6.01±2.132	5.11 ± 2.94	0.0002
Cholesterol	173.72±36.571	192.77±52.121	162.25 ± 29.68	0.0777
TG	187.81±81.318	156.56±53.89	128 .84± 50.36	0.0293
HDL _c	40.46±12.269	39.47±9.536	45.68 ± 8.25	0.3907
LDL _c	94.59±31.237	122.44±47.75	91.09 ± 24.41	0.3907
VLDL	38.22±15.48	31.28±10.78	25.66 ± 10.04	0.0143

The table above indicates there is significant value between GG&AA in (insulin, HOMA-IR& TG), While there is no significant value between GA&AA in Cholesterol.

Table 7. Clinical characteristics of the T2DM groups based on the Dominant model of the HNF4A gene polymorphism SNP rs2144908

Parameters rs2144908 G/A	GG	GA+AA	P-Value
Age	50.94±8.950	48.17±8.117	0.181
BMI	23.91±1.921	25.09±2.757	0.771

FBS	176.44±73.505	178.25±28.835	0.895
Insulin	12.86±9.396	6.262±1.145	<0.001
HOMA_IR	11.86±7.041	2.78±0.770	<0.001
Cholesterol	173.72±36.571	194.56±53.304	0.050
TG	187.81±81.318	151.72±42.796	0.024
HDL_c	40.46±12.269	39.94±9.648	0.832
LDL_c	94.59±31.237	124.26±49.910	0.004
VLDL	38.23±15.848	30.34±8.559	0.015

The Table 7. indicates that for GG & GA+ AA, Insulin, HOMA-IR, Cholesterol, TG, LDL and VLDL are significance values .

4. Discussion

The transcription factor that belongs to the receptor of the nuclear hormone superfamily is known as hepatocyte nuclear factor 4A (HNF4A). The HNF4A is well expressed in the liver and is a member of the nuclear receptor subfamily 2, the member 1 family (NR2A1), and group A. Numerous transcription factors are regulated by HNF4A which controls a number of genes that encode elements of glucose metabolism and insulin production[21-23].It is crucial for hepatocyte development and liver function and is mostly expressed in the liver, gut, and islets of the pancreas[24]. The *HNF4A* gene is situated at 20q12–13.1, where several linkage studies of type 2 diabetes mellitus have been discovered[25]. To maintenance of lipid and glucose homeostasis, *HNF4A* is essential due to its ability to regulate genes involved in insulin, lipid acid, and glucose metabolism [24].

According to certain research, HNF4A controls gene expression in β cells of the pancreas, while the formation and function of β cells in the pancreas are particularly dependent on genes that are regulated by the HNF-4A protein[26] that help to maintain glucose homeostasis and influence the release of insulin and activate the insulin gene directly and indirectly, therefore the *HNF4A* mutations that result in aberrant insulin production not only cause diabetes that develops in adulthood but also maturity-onset diabetes in the young (MODY), which is a condition marked by decreased insulin secretion in carriers of the *HNF4A* mutation, which could be a risk factor of type 2 diabetes[27]. Insulin resistance may be linked with *HNF4A* gene polymorphisms[28]. A study of gene expression in Iraq was conducted in 2023. The results of the investigation found a substantial correlation existed between the biochemical tests being examined and the expression of the HNF4A gene. Therefore, the expression of the HNF4A gene may be a biomarker for seeing when type 2 diabetes mellitus might occur[29].

When a more thorough comparison is made, our results study found that there are notable differences in the codominant, dominant, over dominant, and additive genotypes between the groups based on the *HNF4A* rs2144908 G/A. In Codominant characteristics, it indicates that for GG, GA & AA, insulin, HOMA-IR, TG, and vLDL are significant values, while in Dominant for GG vs GA + AA, the insulin, HOMA-IR, cholesterol, TG, LDL, and VLDL show significant values. The impact of this SNP, rs214908, particularly on the *HNF4A* gene, has been the subject of contradictory research. It could be increase the developing of type 2 diabetes mellitus, according to some research, but it may also protect those who already have the disease.

The present study's findings are consistent with a recent study conducted on the Saudi population by Al-Daghri, N.M., et al., which found that differences in HNF4A were associated with occulted T2DM[30], Also Sudia Arabian researchers Al-Shuhaib et al. discovered a weak correlation between the pathogenic SNPs and the course of type 2 diabetes[31].

Moreover our results align with a number of earlier investigations when the same is indicated, the existence of a possible regulatory element or elements that could raise the hazard of T2DM in Ashkenazi Jews persons in Finland [32] , Danish people [33], Also, the *HNF4A* variants are linked to type 2 diabetes in Scandinavia, and this was according to research on 3,523 Norwegians and a meta-analysis on 11,571 subjects [34]. In a north India study, they found that the *HNF4A* variations rs2144908 were significantly related to T2DM of thin patients[35].In the Chinese community of Hong Kong they found *HNF4A* polymorphism is linked to type 2 diabetes and incident coronary heart disease[36]. T2DM genome-wide studies were analyzed in a large meta-analysis of 6,952 T2D patients and 11,865 controls in East Asia (Korea, China, and Japan) with Finnish people. The ability to identify relationships in these populations may be impacted by the fact that East Asians and Europeans have different allele frequencies.

Additionally, there are significant differences in T2D epidemiology between East Asian and European populations. Lower average BMIs are frequently associated with increased diabetes incidence among East Asians[37] The pathophysiology of T2DM in Europeans and East Asians may include distinct mechanisms [38]. The polymorphism of the gene also affects Taiwanese Population[39].

It was not possible to recreate *HNF4A* SNP associations to T2DM in the French peoples[40],Also not found in American[41] or in Thai people[40]

5. Conclusions

The results showed that SNP rs2144908 G/A was found in Iraqi patients; so our findings indicate that the risk of T2DM will be increased by genetic polymorphism in the *HNF4A*.

However, the small sample size or deficiency of consistency in a lot of recent clinical translation, application, and lack of consistent regulations governing research across cultures and geographical areas with the increased research costs and the difficulty of diagnosing illnesses and determining prognoses and cost-effectiveness, all of these conditions become more challenging for this work, but our result may alter the process of diagnosing and treating type 2 diabetes across Iraq, creating new opportunities for improved patient care and results. So it is recommended to study this gene deeply by prospective studies being carried out with metabolically healthy participants to evaluate the examined SNPs' actual significance as a risk for T2DM and prediabetes disease.

Abbreviation

CHOL	Cholesterol
FBS	Fasting Blood Sugar
HDL	High Density Lipoprotein
HNF4A	Hepatic Nuclear Factor 4A
HOMA-IR	Homeostatic Model Assessment for Insulin Resistant
LDL	Low Density Lipoprotein
SNP	Single Nucleotide Polymorphism
T2DM	Type 2 Diabetes Mellitus
TG	Triglyceride
VLDL	Very Low Density Lipoprotein
WHO	World Health Organization's

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