# Evaluation of the Impact of the WFS1 Gene Rs10010131 (A/G) Variant on the Incidence of Type 2 Diabetes in Iraqi Arab Patients

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## Abstract

Several recent studies have examined the association between a group of genes and the incidence of diabetes mellitus. Some of these have focused particularly on the link between allelic variations of the Wolfram syndrome gene (WFS1) and insulin secretion, insulin sensitivity, and the subsequent risk of hyperglycemia and type 2 diabetes (T2D). While it is obvious that WFS1 mutations induce diabetes, the genetic mechanism for the link between common WFS1 variations and T2D remains unknown. This study was to investigate the association between WFS1 gene polymorphism (rs10010131 A/G) and T2D in Iraqi Arab patients, and particularly whether these effects are more profound for obese patients than for other patients. A case-control study of 200 subjects (100 T2DM and 100 controls) was used to investigate the above relationships. Blood samples were collected and RT-PCR assay was used to genotype WFS1 rs10010131 A/G, making use of newly-designed primers. A significant difference between obese participants in the control and patient sample groups in terms of codominant genotype, dominant genotype, and additive genotype. In addition, TG and VLDL showed a significant difference between AA vs AG and AA vs GG, a negative correlation between F. G and LDL VLDL and between LDL, HDL, and TG, between insulin and HOMA-IR with LDL, HDL. WFS1 gene polymorphism (rs10010131) A/G is associated with a heightened risk of T2DM, particularly in obese T2DM patients in Iraqi Arab patients.

Keywords: WFS1 gene, rs10010131, obese, type 2 diabetes

## 1. Introduction

Noncommunicable diseases (NCDs) accounted for 74 percent of global deaths in 2019, according to the World Health Organization (WHO), with diabetes accounting for 1.6 million deaths, making it the ninth greatest cause of death. According to the International Diabetes Federation (IDF), diabetes affects 8.8% of the adult population, with males (9.6%) having slightly higher rates than women (9.0%) [1]. Iraq ranks 51 in the world, with an age-adjusted death rate of 43.40 per 100,000 people [2].

Diabetes Mellitus (DM) is a chronic disorder in which the pancreas fails to produce enough insulin or has reduced responsiveness to insulin, resulting in a rise in blood glucose levels [3, 4]. T2D has complicated pathogenesis. Elevated glucose levels are caused by three different pathways. Insulin resistance causes skeletal muscle, adipose tissue, and the liver to use less glucose peripherally. Hyperglycaemia is caused by excessive hepatic glucose release as a result of pancreatic islet dysfunction, which is coupled with defective beta-cell insulin release and excess glucagon release from the alpha cells. Furthermore, decreased glucose absorption in the periphery is linked to lower insulin release. With time, the condition worsens as glucotoxicity, inflammation and oxidative stress cause further beta-cell malfunction and insulin resistance [5].

When T2DM is combined with obesity it represents a glycolipid metabolism condition. Treatment of hypoglycemia in this context requires the combination of multiple drugs and this increases the incidence of negative side effects and decreases treatment compliance over time [6]. Processes involving glucotoxicity and/or lipotoxicity, as well as genetic and other variables, are all likely to play a role [7].

WFS1 is one of many genes are involved in T2D [8-13]. The gene codes the protein wolframin, among others, a transmembrane protein involved in calcium regulation in the endoplasmic reticulum and which is used in the pancreas, brain, heart, bones, muscles, lungs, liver, and kidneys [14]. WFS1 is found on the short arm of chromosome 4 at position 16.1 (4p16.1) [15] and encodes a 890-amino-acid protein with nine putative transmembrane segments [16]. Bi-allelic mutations in the WFS1 gene are the major cause of Wolfram syndrome, a rare neurological condition characterized by juvenile-onset diabetes mellitus, optic nerve atrophy, diabetes insipidus, and deafness. The Wolfram syndrome 1 (WFS1) gene was discovered to be the principal causal locus [17]. In that context, several single nucleotide polymorphisms (SNPs) in WFS1 have been found to have substantial correlations with T2DM, with SNP rs10010131 being the strongest [18, 19].

Our research was carried out for several reasons: first, because of the importance of this gene and the impact of obesity on diabetes; second, because, to the best of our knowledge, there are no published studies in Iraq that illustrate the gene's association to Iraqi Arab patients with T2DM.

### Study subjects

A case-control study was devised with 200 subjects (100 T2DM and 100 controls) to assess the relationship between SNP rs10010131 A/G of the WFS1 gene and the incidence of T2DM. One hundred T2DM patients were recruited from patients attending the Al-Shamiya General Hospital in Diwaniyah and

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divided into fifty categorized as obese (twenty male and thirty female), and fifty non-obese (thirty male and twenty female), The control sample, meanwhile, were randomly selected from the general public as well from co-workers and the relatives and of those attending the hospital for a check-up. These were again divided into obese (twenty-four male; twenty-six female) and non-obese (twenty-five male; twenty-five female) groups. The ADA standard was used to categorize T2DM as FPG 126 mg/dl, 2-hPG (2 h Plasma Glucose) 200 mg/dl, or RBG (Random Blood Sugar) 200 mg/dl.

### DNA extraction and genotyping

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

### Primers used in this study

Real time PCR was used to genotype rs10010131A/G, utilizing primers specifically designed for this study in the UGene laboratory, as listed in Table 1 below.

The online server Primer3 was used for this purpose with some modifications.

**Table. 1 The primer sequence for PCR amplification of WFS1 gene polymorphism rs10010131 A/G**

| Target gene | Primer name | Sequence (5’-3’) | Ta (°C) | Product size | Reference | Accession number |
|---|---|---|---|---|---|---|
| WFS 1 | F | AGGCAGGGCACACAAGGC | 56 | 99 bp | UGene lab | NG_011700.1 |
| | R | CCAGGTCGGTCTCGGAGGA | | | | |
| | Allele G | FAM-5'-TTGACCACATCCTGTCCCTCAGGCATC-3'-BHQ1 | 60 | | | |
| | Allele A | HEX-5'-TTGACCACATCCTATCCCTCAGGCATC-3'-BHQ1 | 60 | | | |

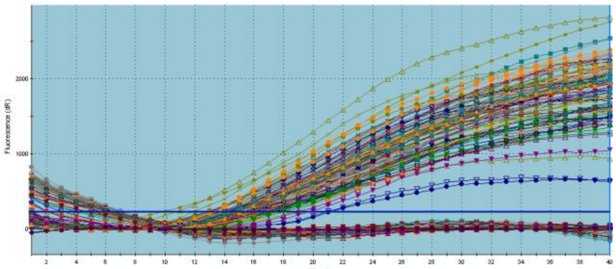


*Fig.1 Real-time PCR amplification curve for the rs10010131 polymorphism of the WF1 gene. The zero line is the threshold line, the X-axis refers to the number of thermal cycles, while the Y-axis refers to the fluorescent intensity.*

### *Real-Time PCR assay*

All the samples were used in the implementation of the conventional Real-Time PCR. One pair of specific primers was used to amplify the conserved region of the WFS1 gene. DNA was extracted from peripheral blood leukocytes using a commercial kit. The WFS1 and rs10010131 A/G polymorphisms were genotyped using primers and probes specific to these SNPs. Reactions were conducted in 0.2 µl wells, in a total 25 ml volume using 2 ng of genomic DNA and a GoTaq® Probe qPCR Master Mix (Promega, USA). The wells were then positioned in a thermal cycler (Stratagene, USA) and heated for 10 min at 95 °C, followed by 40 cycles of 95 °C for 15 s and 60 °C for 1 min. The genotyping success rate was better than 95%, with a calculated error rate based on PCR duplicates of less than 1%.

### Statistical analysis

Although the initial sample comprised one hundred healthy subjects and one hundred patients with T2DM, the analysis was conducted on one hundred healthy and ninety-seven T2DM subjects, since three samples failed to give any results, possibly due to denaturation of their nucleic acids during the thawing/freezing process. The data was analyzed using SPSS v.20.0 software (PASW Statistics, Journal Preproof 8 SPSS Inc., Chicago, IL, USA). The t-test and the Chi-square (χ2) test were used to analyze all the clinical and demographic data, which were provided as mean ± SE. SNP-Analyser version 1.15 ga basic analysis ($p > 0.05$) was performed until the Hardy-Weinberg equilibrium was reached using the frequency of the desired gene in healthy people as the basis. ANOVA was performed between the rs10010131 polymorphism and T2DM details. The odds ratio (OR) was used to compare the presence of genotypes between healthy participants and patients, with a 95% confidence interval (95% CI). A p-value of less than 0.05 was judged as significant in the comparative analysis.

## 2. Results

The present study included two hundred individuals: one hundred controls and one hundred patients divided into four groups.

G1: Control obese
G2: Control non-obese
G3: Patient obese
G4: Patient non-obese

T2DM patients and controls had mean ages of (48.69 ± 7.21) and (46.29 ± 7.31), respectively. Obese and non-obese T2DM patients had mean BMI of (32.43 ± 2.57) and (23.76 ± 1.17), respectively. Obese and non-obese controls had mean BMI of (32.72 ± 1.91) and (23.95 ± 1.03), respectively. Our results showed the statistically significant differences in diabetes-related biochemical and clinical characteristics between control obese / non-obese and T2DM obese / non-obese individuals, as set out in table 2.

**Table. 2 Summary of the clinical and biochemical parameters of T2DM and control groups**

| Groups<br>Parameters | Mean | SE | Compared Groups | | Sign. |
|---|---|---|---|---|---|
| FG<br>G1 | 103.280 | 2.4137 | 1 | 2 | 0.76 |
| | | | | 3 | 0.0001 |
| | | | | 4 | 0.0001 |
| G2 | 99.861 | 2.3525 | 2 | 1 | 0.76 |

| | | | | | |
|---|---|---|---|---|---|
| | | | | 3 | 0.0001 |
| | | | | 4 | 0.0001 |
| G3 | 183.980 | 9.0887 | 3 | 1 | 0.0001 |
| | | | | 2 | 0.0001 |
| | | | | 4 | 0.79 |
| G4 | 181.100 | 12.6923 | 4 | 1 | 0.0001 |
| | | | | 2 | 0.0001 |
| | | | | 3 | 0.79 |
| Chol<br>G1 | 160.600 | 5.1589 | 1 | 2 | 0.33 |
| | | | | 3 | 0.1 |
| | | | | 4 | 0.01 |
| G2 | 152.894 | 4.4666 | 2 | 1 | 0.33 |
| | | | | 3 | 0.01 |
| | | | | 4 | 0.001 |
| G3 | 173.560 | 5.5169 | 3 | 1 | 0.1 |
| | | | | 2 | 0.01 |
| | | | | 4 | 0.43 |
| G4 | 179.760 | 7.0662 | 4 | 1 | 0.01 |
| | | | | 2 | 0.001 |
| | | | | 3 | 0.43 |
| TG<br>G1 | 119.640 | 7.5277 | 1 | 2 | 0.66 |
| | | | | 3 | 0.0001 |
| | | | | 4 | 0.0001 |
| G2 | 111.819 | 7.7756 | 2 | 1 | 0.66 |
| | | | | 3 | 0.0001 |
| | | | | 4 | 0.0001 |
| G3 | 227.320 | 14.3021 | 3 | 1 | 0.0001 |
| | | | | 2 | 0.0001 |
| | | | | 4 | 0.44 |
| G4 | 241.079 | 17.7252 | 4 | 1 | 0.0001 |
| | | | | 2 | 0.0001 |
| | | | | 3 | 0.44 |
| HDL<br>G1 | 38.680 | 1.05509 | 1 | 2 | 0.35 |
| | | | | 3 | 0.51 |
| | | | | 4 | 0.93 |
| G2 | 40.741 | 1.6093 | 2 | 1 | 0.35 |
| | | | | 3 | 0.004 |
| | | | | 4 | 0.31 |
| G3 | 34.340 | 1.3524 | 3 | 1 | 0.51 |
| | | | | 2 | 0.004 |
| | | | | 4 | 0.06 |
| G4 | 38.501 | 1.7155 | 4 | 1 | 0.93 |
| | | | | 2 | 0.31 |
| | | | | 3 | 0.06 |
| LDL<br>G1 | 98.220 | 4.0455 | 1 | 2 | 0.21 |
| | | | | 3 | 0.29 |
| | | | | 4 | 0.009 |
| G2 | 89.258 | 2.9969 | 2 | 1 | 0.21 |
| | | | | 3 | 0.85 |
| | | | | 4 | 0.16 |
| G3 | 90.620 | 5.4995 | 3 | 1 | 0.29 |
| | | | | 2 | 0.85 |
| | | | | 4 | 0.11 |
| G4 | 79.086 | 6.9543 | 4 | 1 | 0.009 |
| | | | | 2 | 0.16 |
| | | | | 3 | 0.11 |
| VLDL<br>G1 | 24.328 | 1.5371 | 1 | 2 | 0.46 |
| | | | | 3 | 0.0001 |
| | | | | 4 | 0.0001 |
| G2 | 22.119 | 1.5350 | 2 | 1 | 0.46 |
| | | | | 3 | 0.0001 |
| | | | | 4 | 0.0001 |
| G3 | 42.291 | 2.5807 | 3 | 1 | 0.0001 |
| | | | | 2 | 0.0001 |
| | | | | 4 | 0.11 |

| | | | | | |
|---|---|---|---|---|---|
| G4 | 37.540 | 2.6118 | 4 | 1 | 0.0001 |
| | | | | 2 | 0.0001 |
| | | | | 3 | 0.11 |
| Insulin<br>G1 | 86.548 | 11.9016 | 1 | 2 | 0.56 |
| | | | | 3 | 0.001 |
| | | | | 4 | 0.58 |
| G2 | 74.778 | 9.5423 | 2 | 1 | 0.56 |
| | | | | 3 | 0.0001 |
| | | | | 4 | 0.26 |
| G3 | 154.224 | 21.3020 | 3 | 1 | 0.001 |
| | | | | 2 | 0.0001 |
| | | | | 4 | 0.007 |
| G4 | 97.738 | 12.7542 | 4 | 1 | 0.58 |
| | | | | 2 | 0.26 |
| | | | | 3 | 0.007 |
| HOMA-IR<br>G1 | 25.4242 | 2.90014 | 1 | 2 | 0.72 |
| | | | | 3 | 0.0001 |
| | | | | 4 | 0.012 |
| G2 | 23.5542 | 2.82261 | 2 | 1 | 0.72 |
| | | | | 3 | 0.0001 |
| | | | | 4 | 0.04 |
| G3 | 67.6319 | 8.71768 | 3 | 1 | 0.0001 |
| | | | | 2 | 0.0001 |
| | | | | 4 | 0.024 |
| G4 | 46.0958 | 8.26734 | 4 | 1 | 0.012 |
| | | | | 2 | 0.04 |
| | | | | 3 | 0.024 |
| HOMA-IR<br>G1 | 25.4242 | 2.90014 | 1 | 2 | 0.72 |

**Table. 3 According to the HWE, SNP rs10010131A/G in the human gene WFS1 study of the genotype of the control subjects.**

| Genotype of control | Observed | Expected | Difference | X2 | P value |
|---|---|---|---|---|---|
| AA Reference | 26 | 18.94 | 7.06 | 8.3 | <0.003 |
| AG Heterozygote | 35 | 49.14 | 14.14 | | |
| GG Recessive | 39 | 31.92 | 7.08 | | |
| A allele% | 43.5 | | | | |
| G allele% | 56.5 | | | | |

The genotype frequencies of gene WFS1SNP rs10010131A/G are not consistent with the Hardy-Weinberg equilibrium for controlling obese and control non-obese samples.

**Table. 4 According to the HWE, SNP rs10010131 A/G in the human gene WFS1 study of the genotype of the patient subjects.**

| Genotype of patients | Observed | Expected | Difference | X2 | P value |
|---|---|---|---|---|---|
| AA Reference | 5 | 10.56 | 5.56 | 6.5 | 0.01 |
| AG Heterozygote | 54 | 42.88 | 11.12 | | |
| GG Recessive | 38 | 43.56 | 5.56 | | |
| A allele% | 33 | | | | |
| G allele% | 67 | | | | |

The genotype frequencies of gene WFS1SNP rs10010131A/G are not consistent with the Hardy-Weinberg equilibrium for patient obese and patient non-obese samples.

**Table. 5 Genotype of rs10010131 A/G polymorphism of total healthy subjects and total patients group.**

| rs10010131 A/G | Control N=100 | Patients N=100 | Crude OR (CI 95%) P value |
|---|---|---|---|
| Codominant | | | |
| AA | 26 | 5 | -- |
| AG | 35 | 54 | 8.02<br>(2.82-22.87)<br>0.0001 |
| GG | 39 | 38 | 5.1<br>(1.76-14.57)<br>0.003 |
| Dominant | | | |
| AG+GG | 74 | 92 | 6.5<br>(2.36-17.66)<br>0.0001 |
| Recessive | | | |
| AA+AG | 60 | 58 | -- |
| GG | 40 | 39 | 1.01<br>(0.5-1.78)<br>0.9 |
| Additive | | | |
| 2(AA)+AG | 87 | 64 | -- |
| 2(GG)+AG | 113 | 130 | 1.5<br>(1.0383 to 2.3554)<br>0.03 |

The table shows the comparison between the control and T2DM groups. Significant variation was observed in codominant, dominant, and additive models, respectively.

**Table 6 Genotype of rs10010131 A/G polymorphism of control obese and patient obese groups.**

| rs10010131 A/G | Control Obese N=50 | Patients Obese N=48 | Crude OR (CI 95%) P value | Adjusted OR (CI 95%) P value |
|---|---|---|---|---|

| | | | | |
|---|---|---|---|---|
| Codominant | | | | |
| AA | 19 | 3 | -- | -- |
| AG | 13 | 25 | 3.1<br>(1.9-5.03)<br>0.0001 | 3.2<br>(1.8-5.5)<br>0.0001 |
| GG | 18 | 20 | 2.4<br>(1.5-3.91)<br>0.0001 | 2.4<br>(1.4-4.21)<br>0.002 |
| Dominant | | | | |
| AG+GG | 31 | 45 | 6.4<br>(2.4-17.6)<br>0.0001 | 6.2<br>(2.2-17.2)<br>0.0001 |
| Recessive | | | | |
| AA+AG | 32 | 28 | -- | -- |
| GG | 18 | 19 | 1.00<br>(0.57-1.78)<br>0.97 | 1.02<br>(0.57-1.8)<br>0.94 |
| Additive | | | | |
| 2(AA)+AG | 51 | 31 | -- | -- |
| 2(GG)+AG | 49 | 65 | 2.2<br>(1.2215 - 3.8989)<br>0.008 | |

The table shows the comparison between the control obese and T2DM obese groups. Significant variation was observed in codominant, dominant, and additive models, respectively.

**Table. 7 Genotype of the rs10010131 A/G polymorphism of control non-obese and patient non-obese**

| 10010131 A/G | Control non Obese N=50 | Patients non Obese N=49 | Crude OR (CI 95%) P value | Adjusted OR (CI 95%) P value |
|---|---|---|---|---|
| Codominant | | | | |
| AA | 7 | 2 | - | |
| AG | 22 | 29 | 2.15<br>(0.94-4.9)<br>0.07 | 0.9<br>(0.05-14.2)<br>0.9 |
| GG | 21 | 18 | 1.7<br>(0.7-4.04)<br>0.2 | 0.68<br>(0.04-10.9)<br>0.78 |
| Dominant | | | | |
| AG+GG | 43 | 47 | 3.83<br>(0.75-19.43)<br>0.11 | 0.59<br>(0.1-18.5)<br>0.9 |
| Recessive | | | | |
| AA+AG | 29 | 31 | - | - |
| GG | 21 | 29 | 0.81<br>(0.36-1.79)<br>0.59 | 0.14<br>(0.002-10.3)<br>0.37 |
| Additive | | | | |
| 2(AA)+AG | 36 | 33 | - | |
| 2(GG)+AG | 64 | 65 | 1.11<br>(0.6172 - 1.9888)<br>0.73 | |

The table shows the comparison between the control non-obese and T2DM non-obese groups. No significant variation was observed in the codominant, dominant, recessive, and additive models, respectively.

**Table. 8 Clinical parameters of T2DM groups according to WFS1 gene polymorphism SNP rs10010131 genotype (codominant model)**

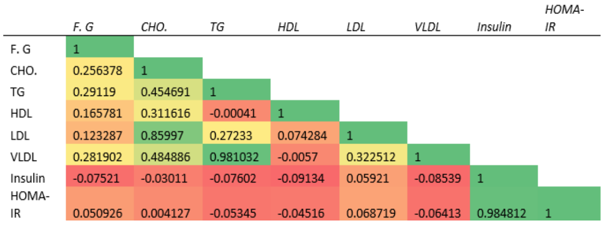
| Clinical parameter | AA N=5 | AG N=54 | GG N=38 | P value |
|---|---|---|---|---|
| Age | 51.0±2.63 | 45.3±0.97 | 47.0±1.2 | 0.182 |
| BMI | 28.8±1.8 | 27.9±0.6 | 28.5±0.77 | 0.816 |
| FG | 102.4±2.6 | 99.2±2.3 | 102.5±2.6 | 0.6 |
| Cho | 184.8±29.0 | 152.4±4.5 | 157.4±4.9 | 0.136 |
| TG | 180.0±50.4 | 110.5±6.7 | 113.8±7.63 | 0.022<br>AAvs AG0.006<br>AA vs GG 0.01 |
| HDL | 48.8±7.1 | 39.3±1.6 | 39.2±1.6 | 0.185 |
| LDL | 104.0 ±15.5 | 92.1±3.5 | 93.7±4.0 | 0.609 |
| VLDL | 36.0±10.1 | 22.2±1.3 | 23.2±1.5 | 0.023<br>AAvs AG0.006<br>AA vs GG 0.01 |
| Insulin | 58.5±18.5 | 91.2±12.6 | 67.81±8.3 | 0.293 |
| HOMA-IR | 20.55±8.2 | 26.3±3.04 | 21.2±2.8 | 0.535 |

This table shows that TG and VLDL exhibited significant differences between AA vs AG and AA vs GG.

**Table. 9 Clinical parameters T2DM groups according to WFS1 gene polymorphism SNP rs10010131genotype (dominant model)**

| Clinical parameter | AA N=5 | AG+GG N=92 | P value |
|---|---|---|---|
| Age | 51.0±2.63 | 46.3±0.7 | 0.61 |
| BMI | 28.8±1.8 | 28.2±.05 | 0.872 |
| FG | 102.4±2.6 | 100.6±1.7 | 0.926 |
| Cho | 184.8±29.0 | 156.0±3.7 | 0.795 |
| TG | 180.0±50.4 | 115.7±5.7 | 0.769 |
| HDL | 48.8±7.1 | 39.1±1.13 | 0.024 |
| LDL | 104.0 ±15.5 | 93.6±2.7 | 0.623 |
| VLDL | 36.0±10.1 | 23.3±1.2 | 0.753 |
| Insulin | 58.5±18.5 | 81.6±8.2 | 0.514 |
| HOMA-IR | 20.4±8.3 | 24.4±2.14 | 0.67 |

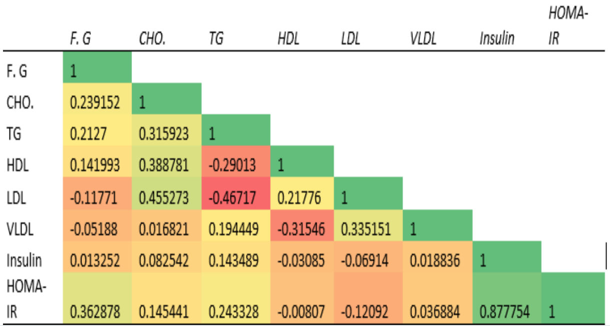
The table shows appear HDL significant at AA&GA+AA



| | F. G | CHO. | TG | HDL | LDL | VLDL | Insulin | HOMA-IR |
|---|---|---|---|---|---|---|---|---|
| F. G | 1 | | | | | | | |
| CHO. | 0.256378 | 1 | | | | | | |
| TG | 0.29119 | 0.454691 | 1 | | | | | |
| HDL | 0.165781 | 0.311616 | -0.00041 | 1 | | | | |
| LDL | 0.123287 | 0.85997 | 0.27233 | 0.074284 | 1 | | | |
| VLDL | 0.281902 | 0.484886 | 0.981032 | -0.0057 | 0.322512 | 1 | | |
| Insulin | -0.07521 | -0.03011 | -0.07602 | -0.09134 | 0.05921 | -0.08539 | 1 | |
| HOMA-IR | 0.050926 | 0.004127 | -0.05345 | -0.04516 | 0.068719 | -0.06413 | 0.984812 | 1 |

*Fig .2 Correlation of clinical parameters for control samples*

The above figure shows the correlation between the lipid profile, FG, insulin, and HOMA–IR for control samples. The results indicate a negative correlation between insulin and HOMA-IR and F. G, CHO, TG, VLDL.
(A green colour indicates a safe level, while a red colour indicates a dangerous level).



| | F. G | CHO. | TG | HDL | LDL | VLDL | Insulin | HOMA-IR |
|---|---|---|---|---|---|---|---|---|
| F. G | 1 | | | | | | | |
| CHO. | 0.239152 | 1 | | | | | | |
| TG | 0.2127 | 0.315923 | 1 | | | | | |
| HDL | 0.141993 | 0.388781 | -0.29013 | 1 | | | | |
| LDL | -0.11771 | 0.455273 | -0.46717 | 0.21776 | 1 | | | |
| VLDL | -0.05188 | 0.016821 | 0.194449 | -0.31546 | 0.335151 | 1 | | |
| Insulin | 0.013252 | 0.082542 | 0.143489 | -0.03085 | -0.06914 | 0.018836 | 1 | |
| HOMA-IR | 0.362878 | 0.145441 | 0.243328 | -0.00807 | -0.12092 | 0.036884 | 0.877754 | 1 |

*Fig.3 Correlation of clinical parameters for patient samples.*

The above figure shows the correlation between the lipid profile, FG, insulin, and HOMA–IR for patient samples. The results indicate a negative correlation between F. G and LDL, VLDL, and between insulin / HOMA-IR and LDL, HDL (A green color indicates a safe level, while red color indicates a dangerous level).

## 3. Discussion

Obesity has tripled in prevalence worldwide since 1975 and is associated with the development of T2DM [20], along with a wide range of other comorbidities. In obesity, excess energy, coupled with the release of damage-associated molecular patterns from dead adipocytes, promotes chronic adipose tissue inflammation that correlates with insulin resistance [21]. Furthermore, limited insulin receptor activation (a hallmark of T2DM) is associated with the overproduction of the adipocytokine tumor necrosis factor (TNF) [22] and the adipokine leptin [23]. These were the first factors linking lymphocytes to all inflammatory aspects of T2DM and other complications of obesity. Obesity-related T2D may further increase the risk of developing comorbidities like cardiovascular diseases, hypertension, neurological and neuropsychiatric illness, and autoimmune diseases [24, 25].

T2DM is the most common progressive metabolic disorder with complex molecular pathology involving defective lipid metabolism, insulin resistance, and beta-cell functioning. Although previous investigations have reported various potential molecular markers linked with the progression of obesity and T2DM, the key common genes, and the molecular mechanisms underlying its pathogenicity remain elusive [26].

Various studies conducted in a range of ethnic populations have shown that mutations in WFS1 are associated with T2DM [25]. Based on the available data, the WFS1 gene is connected with Wolfram syndrome (WS), also known as diabetes insipidus, insulin-deficient diabetes mellitus, optic atrophy, and deafness [27]. Type II diabetes risk markers were identified from published Genome-wide association studies (GWAS) and include many markers, such as rs10010131/WFS1[28, 29], that are associated with reduced insulin secretion [30].

In individuals with abnormal glucose regulation, defined as IFG, IGT, or screen-detected and untreated T2DM, the reported WFS1 diabetes risk alleles are significantly associated with impaired insulin release as assessed by insulinogenic index (6.5% per allele), whereas the same alleles in glucose-tolerant individuals were associated with increased fasting serum insulin and HOMA-IR. To date, two studies [31, 32] have investigated the association between WFS1 variants and T2DM suggested by Sandhu et al. [33]. All T2DM patients were confirmed to be overweight subjects, and previous studies recorded obesity as one of the risk factors for T2DM as well as Gestational Diabetes Mellitus (GDM) [34].

Our results show a significant association between rs10010131 polymorphism controls and patients and a significant difference between control obese and patient obese genotypes for the codominant genotype, dominant genotype, and additive genotype. Based on our results, it can be concluded that the rs10010131 polymorphism is a risk factor for T2DM in Iraqi Arab populations. This agrees with the previous research cited above but extends it to the Iraqi Arab context. Four SNPs (rs10010131, rs6446482, rs752854, and rs734312 (H611R)) in the WFS1 gene have been demonstrated to be associated with T2DM in Caucasians [31], but polymorphisms rs734312 and rs10010131 were found to have a negative relationship with T2DM in meta-analysis studies [35].

We present compelling evidence for a link between WFS1 variation and the risk of T2DM. The WFS1 gene encodes for wolframin, a membrane glycoprotein that helps the endoplasmic reticulum maintain calcium homeostasis. Wolfram syndrome is characterized by diabetes insipidus, juvenile-onset non-autoimmune diabetes mellitus, optic atrophy, and deafness, and is caused by mutations in this gene [36, 37]. Depending on the genetic background of the mice, disruption of Wfs1 induces overt diabetes or decreased glucose tolerance [38, 39]. Wolframin deficiency causes pancreatic b cell loss in both humans and animals, probably as a result of an increased endoplasmic reticulum stress response, which leads to increased b cell death [39-43]. As a result, WFS1 is required for insulin-producing pancreatic b cells to survive and function. Chemical improvement of endoplasmic reticulum activity has been proposed as a therapy for the metabolic problems associated with diabetes, based on this research [44].

## 4. Conclusions

The gene WFS1 polymorphism rs10010131 polymorphism significant association between Controls and patients and When a more accurate comparison is made, there is a significant difference between Controls obese and patients obese genotype Depend on our results rs10010131 polymorphism represents risk factors for T2DM.

Declaration of Competing Interest

The authors declare no competing financial interests.

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