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The Impact of CYP2C9 Rs1057910 and rs1799853 Polymorphism on the Effectiveness of Glibenclamide in T2DM Iraqi Patients

Fatima Ridha Shareef[1], Zahra Abdel Al-Kareem Mohammed[2], Shiama Jabbar[2]*
[1] Graduate program of Pharmacology and Toxicology, College of Pharmacy, University of Kerbala, Karbala, Iraq
[2] Department of Pharmacology and Toxicology, College of Pharmacy, University of Kerbala, Karbala, Iraq

***Corresponding Author:** Shaima Jabbar: shaymaa.a@uokerbala.edu.iq.

ABSTRACT

Background: Diabetes mellitus (DM) is a complex metabolic disorder characterized by chronic hyperglycemia and dysregulation of carbohydrate, lipid, and protein metabolism. Glibenclamide remains one of the most commonly prescribed sulfonylureas for the management of type 2 diabetes mellitus (T2DM). However, interindividual variability in therapeutic response has been widely reported. Genetic polymorphisms, particularly in genes regulating glibenclamide pharmacokinetics and pharmacodynamics, are thought to contribute to these differences in drug disposition and clinical efficacy. **Objective:** This study aimed to evaluate the impact of CYP2C9 gene polymorphisms on the therapeutic effectiveness of glibenclamide in Iraqi patients with T2DM Iraqi patients. **Materials and Methods:** A case–control study was conducted in Karbala City, located in central Iraq. The study population comprised 120 patients with a confirmed diagnosis of diabetes mellitus and 100 healthy individuals. Venous blood samples were obtained from all participants for the assessment of glycemic control parameters and for genetic analysis. Sample collection and clinical data acquisition were performed during patients' routine visits to the Imam Hassan Center for Endocrinology and Diabetes in Karbala City. **Results:** The findings of this study demonstrated no statistically significant association between CYP2C9 gene polymorphisms (rs1057910 and rs1799853) and therapeutic response to glibenclamide ($p \geq 0.05$). However, a statistically significant association was observed between patients' glycemic response to glibenclamide therapy and gender across both wild-type and mutant genotype groups ($p \leq 0.05$). **Conclusion:** This study highlights important concerns regarding the role of gender (male/female) in the variation of T2DM patients' response to glibenclamide therapy.

Keywords: *Type2 diabetes mellitus, glibenclamide, CYP2C9 enzyme, polymorphism.*

INTRODUCTION

Diabetes mellitus (DM) is a multifactorial metabolic disorder characterized by chronic hyperglycemia and disturbances in carbohydrate, lipid, and protein metabolism. The condition arises from either insulin deficiency, secondary to progressive β-cell dysfunction, or insulin resistance, or a combination of both, ultimately leading to persistent elevation of blood glucose levels. Chronic hyperglycemia contributes to oxidative cellular damage and dysfunction of multiple organs, including the kidneys, eyes, nerves, liver, heart, and blood vessels (Brison, 2017) .DM encompasses several subtypes, including type 1 DM, type 2

DM (T2DM), gestational diabetes, neonatal diabetes, and secondary diabetes caused by endocrine disorders or prolonged steroid use. In Iraq, the prevalence of T2DM is estimated to range between 8.5% (age-adjusted, International Diabetes Federation) and 13.9%. Despite this high prevalence, understanding the epidemiology of diabetes in Iraq and identifying the most effective therapeutic strategies remain challenging due to the limited number of population-based epidemiological studies and randomized controlled trials (RCTs)(Abusaib et al., 2020).Glibenclamide, a second-generation sulfonylurea, is approved by the U.S. Food and Drug Administration (FDA) for the treatment of T2DM. It effectively reduces blood glucose levels by inhibiting ATP-sensitive potassium (K^+) channels in pancreatic β-cells, leading to cell depolarization and insulin secretion. Additionally, glibenclamide exhibits extra pancreatic effects in the liver, skeletal muscle, cardiac muscle, and smooth muscle through similar mechanisms. Following oral administration, the drug reaches its maximum plasma concentration (C_max) within 1.62–2.09 hours. There is a linear dose–concentration relationship for glibenclamide, with maximum plasma concentrations (C_max) increasing proportionally as the dose rises from 2.5 mg to 20 mg, corresponding to 157.97 ng/mL and 773.61 ng/mL, respectively. Once absorbed, glibenclamide is widely distributed throughout the body, with approximately 99.9% bound to plasma proteins, of which more than 98% is bound to serum albumin (Luzi and Pozza, 1997).

Glibenclamide is primarily metabolized by cytochrome P450 (CYP) enzymes, including CYP2C9, CYP2C19, CYP3A7, and CYP3A5. These metabolic pathways yield several metabolites: 4-trans-hydroxycyclohexyl glibenclamide (M1), 4-cis-hydroxycyclohexyl glibenclamide (M2a), 3-cis-hydroxycyclohexyl glibenclamide (M2b), 3-trans-hydroxycyclohexyl glibenclamide (M3), 2-trans-hydroxycyclohexyl glibenclamide (M4), and ethyl hydroxycyclohexyl glibenclamide (M5). The parent drug and its M1 and M2b metabolites are considered pharmacologically active. The elimination half-life ($t_{1/2}$) of glibenclamide ranges from 4.42 to 8.08 hours, with approximately 50% of the administered dose excreted in the urine and the remaining 50% eliminated via feces (Sonnenblick and Shilo, 1986) . Cytochrome P450 2C9 (CYP2C9) is the most abundant isoenzyme within the CYP2 subfamily, accounting for more than 20% of the total cytochrome P450 content in human liver microsomes. It is responsible for the metabolism of approximately 10–20% of commonly prescribed medications. The CYP2C9 gene displays substantial genetic variability across different racial and ethnic populations. According to the Pharmacogenetic Variation (PharmVar) Consortium, 62 allelic variants of CYP2C9 have been identified to date, the majority of which are single nucleotide polymorphisms (SNPs) capable of altering a single amino acid and consequently affecting the catalytic activity of the expressed enzyme (Liu et al., 2021). The CYP2C9 gene is located on chromosome 10q24 and exhibits polymorphisms within its coding region that give rise to multiple allozymes. In addition to the wild-type allele (CYP2C91), at least five SNPs have been described, producing functionally distinct enzyme variants. Of these, CYP2C9*2 (Arg144Cys) and CYP2C9*3 (Ile359Leu) are the most clinically relevant, as they can significantly modify substrate specificity and catalytic efficiency, potentially altering drug metabolism and therapeutic response to agents such as glibenclamide(Salam et al., 2014) .

MATERIALS AND METHODS

Study Population and Design: A total of 220 participants were enrolled in this study, comprising 100 healthy individuals (47 males and 53 females) and 120 patients with type 2 diabetes mellitus (58 males and 62 females), aged 20–55 years. Recruitment was conducted at the Imam Hassan Center for Endocrinology and Diabetes in Karbala City, Iraq—a city located approximately 100 km (62 miles) southwest of Baghdad with an estimated population of 691,100 as of 2024. The study was carried out between September 2024 and March 2025. All patients had a confirmed diagnosis of T2DM based on established diagnostic criteria. This investigation was designed as a cross-sectional, observational study to evaluate the impact of CYP2C9 genetic polymorphisms on the pharmacological efficacy of glibenclamide in Iraqi patients with T2DM.

Venous blood samples were obtained from overnight-fasted participants who were already receiving glibenclamide therapy. The samples were analyzed for biochemical, hormonal, and genetic parameters. All standard infection control measures were strictly followed during sample collection in the clinical setting.

Sample Collection: Following an overnight fast, venous blood samples were collected from all participants. Each sample was divided into two portions: the first portion (2 mL) was placed in an EDTA tube for glycated hemoglobin (HbA1c) measurement and genomic DNA extraction, while the second portion (3 mL) was placed in a serum-separating gel tube for serum isolation and subsequent hormonal and biochemical analyses.

Biochemical analysis

Glycemic control analysis

Serum insulin concentrations were measured using the Cobas e411 analyzer (Roche Diagnostics) employing a one-step immunoassay based on Chemiluminescent Immunoassay (CIMA) technology with flexible assay protocols (Chemiflex). Glycated hemoglobin (HbA1c) levels were measured from EDTA-anticoagulated whole blood using the Lifotronic H8 automated system, which performs automatic hemolysis before analysis. Other parameters like HOMA-IR (homeostasis model assessment-insulin resistance) and HOMA-B (homeostasis model assessment-beta cell function) levels were calculated by specific equations ("H8.pdf," n.d.): HOMA-IR = [fasting insulin (μU/ml) × fasting plasma glucose (mg/dl)]/ 405, and HOMA-B = 360×fasting insulin (μU/ml)/[fasting plasma glucose (mg/dl) -63]

lipid profile analysis:

Serum total cholesterol (TC), triglycerides (TG), high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C) levels were quantified using standardized enzymatic colorimetric methods on the BS240Pro automated chemistry analyzer. Total cholesterol was determined via the cholesterol esterase–cholesterol oxidase–peroxidase sequence, producing a chromogenic end-product with absorbance measured at 512 nm (States and Accounting, 1994). Triglycerides were measured through enzymatic hydrolysis by lipase, followed by coupled oxidation reactions generating hydrogen peroxide, which was quantified colorimetrically at 500 nm. HDL-C was estimated using a homogeneous enzymatic selective protection method, in which non-HDL lipoproteins (LDL, VLDL, chylomicrons) were selectively masked, allowing specific quantification of HDL-C through enzymatic oxidation and colorimetric detection at 583 nm (Yanai and Tada, 2018). LDL-C levels were measured using a similar homogeneous selective method with surfactants that specifically solubilize LDL, followed by enzymatic determination of cholesterol content and photometric measurement of the resulting chromogen (Direct, n.d.).

Kidney and liver function analysis

Renal function was evaluated by measuring serum urea and creatinine levels using standardized enzymatic quantitative methods on the Monarch/240 analyzer. Urea was determined by urease-mediated hydrolysis followed by glutamate dehydrogenase (GLDH)-catalyzed reactions, with the rate of NADH consumption being proportional to urea concentration. Serum creatinine was quantified through a multi-step enzymatic process involving creatinase, sarcosine oxidase, and peroxidase, with the chromogenic end-product measured spectrophotometrically at 546 nm. Hepatic function was assessed by measuring alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities using IFCC-recommended methods on the same system. ALT activity was determined through coupled enzymatic reactions that convert alanine and 2-oxoglutarate to pyruvate and glutamate, with subsequent conversion of pyruvate to lactate via lactate dehydrogenase (LDH) and measurement of NADH consumption. AST activity was similarly quantified by catalyzing the conversion of aspartate and 2-oxoglutarate to oxaloacetate and glutamate, followed by reduction of oxaloacetate to malate via malate dehydrogenase (MDH), with NADH

oxidation serving as the measurable endpoint (D'sa and Lakshmi, 2010).

Genetic analysis: Genomic DNA was extracted from peripheral blood samples using the Geneaid™ DNA Isolation Kit, which employs a gentle reagent-based precipitation method for the isolation of high–molecular–weight genomic DNA(Geneaid Biotech Ltd., 2008). Genotyping of CYP2C9*2 (rs1799853) and CYP2C9*3 (rs1057910) polymorphisms was carried out using polymerase chain reaction (PCR) with allele-specific primers, as detailed in Tables 1 and Table2.

PCR Amplification and Electrophoresis:
Polymerase chain reaction (PCR) conditions were optimized for both target polymorphisms. For CYP2C9*2 (rs1799853), the PCR protocol consisted of an initial denaturation step at 95 °C for 4 minutes, followed by 35 amplification cycles comprising denaturation at 95 °C for 40 seconds, annealing at 68 °C for 30 seconds, and extension at 72 °C for 30 seconds, with a final extension at 72 °C for 5 minutes. For CYP2C9*3 (rs1057910), the same cycling conditions were applied except for the annealing temperature, which was set at 67 °C.PCR products were separated by agarose gel electrophoresis on 1.5% agarose gels at 70 V for 90 minutes (Westermeier, 2005). DNA bands were visualized using an ultraviolet transilluminator and documented by digital photography, as illustrated in Figure1 and Figure2.

Statistical analysis:
Data were analyzed using IBM SPSS Statistics software, version 26.0 (IBM Corp., Armonk, NY, USA). Associations between categorical variables were examined using the Chi-square test or Fisher's exact test, where appropriate. Logistic regression analysis was performed to estimate odds ratios (ORs) and corresponding 95% confidence intervals (CIs) for predictors of treatment response. Bivariate correlation analysis was employed to assess the relationship between CYP2C9 single-nucleotide polymorphisms (SNPs) and diabetes-related clinical and biochemical parameters. A two-tailed p-value ≤ 0.05 was considered statistically significant.

RESULT

Analysis of Glycemic Control Parameters of Healthy Control Group and Type2 Diabetic (T2DM) patients Group. Data presented in Table3 demonstrated statistically significant differences in glycemic control parameters — including fasting blood glucose (FBG), glycated hemoglobin (HbA1c), HOMA-IR, and HOMA-B — between the two study groups ($p < 0.0001$). In contrast, no significant difference was observed in serum insulin concentrations between the groups ($p = 0.2057$). Gender-based analysis revealed noteworthy findings. HbA1c and FBG levels were significantly higher in female T2DM patients compared with male patients ($p = 0.0003$ for HbA1c and $p = 0.0381$ for FBG). Additionally, significant differences were observed between healthy males and diabetic females, as well as between diabetic males and healthy females ($p < 0.0001$ for both comparisons), as illustrated in Figure3A and Figure2B. HOMA-IR also showed significant variation between female and male T2DM patients ($p = 0.0010$), with additional significant differences noted between healthy males and diabetic females ($p = 0.0043$) and between diabetic males and healthy females ($p < 0.0001$), as shown in Figure3C. Similarly, HOMA-B demonstrated a significant difference between diabetic males and healthy females ($p = 0.0001$), as illustrated in Figure3D. No statistically significant difference in serum insulin levels was observed between males and females, as shown in Figure3E.

Analysis of lipid profile of healthy control group and T2DM studied groups

Data presented in Figure4 showed total cholesterol levels did not differ significantly between the healthy and T2DM groups, nor between males and females, as presented in Figure4A. Statistically significant differences in lipid profile parameters — including triglycerides (TG), high-density lipoprotein cholesterol

(HDL-C), and low-density lipoprotein cholesterol (LDL-C) — between healthy controls and T2DM patients ($p < 0.0001$). Notably, HDL-C levels were significantly different between females and males within the T2DM group ($p = 0.0484$). Moreover, significant differences in both TG and HDL-C levels were observed between healthy females and diabetic males, as well as between diabetic males and healthy females ($p < 0.0001$ for both comparisons), as illustrated in Figures4B-C. Regarding LDL-C, a significant difference was detected between diabetic males and healthy females ($p = 0.0106$), and between healthy males and diabetic females ($p = 0.0078$), as shown in Figure4D.

Analysis of liver function enzymes of healthy control subjects and patients with T2DM

Data presented in Table5 demonstrated a statistically significant elevation in alanine aminotransferase (ALT) levels among T2DM patients compared with healthy controls ($p < 0.0001$). Furthermore, a significant difference in ALT levels was observed between female and male participants within the T2DM group ($p = 0.0274$), as illustrated in Figure5A. In contrast, no statistically significant difference in aspartate aminotransferase (AST) levels was detected between T2DM patients and healthy controls, nor between males and females, as shown in Figure5B.

Comparing of kidney function parameters of healthy people to T2DM patients:

There was no difference in serum urea levels, between the overall healthy and T2DM groups ($p = 0.4705$). However, a significant difference was observed between healthy and diabetic females ($p = 0.0038$), with healthy females showing higher mean urea concentrations (32.51 ± 7.47 mg/dL) compared with diabetic females (27.36 ± 7.62 mg/dL), as presented in Figure6A and Table6.

We found a statistically significant difference in serum creatinine levels between healthy controls and T2DM patients ($p = 0.0122$). A highly significant difference was also observed between females and males within the T2DM group ($p < 0.0001$), as well as between healthy and diabetic females ($p < 0.0001$). Interestingly, healthy females exhibited higher mean creatinine levels (0.909 ± 0.22 mg/dL) compared with diabetic females (0.590 ± 0.174 mg/dL), see Figure6B.

Genotype and allele frequency analysis of CYP2C9*3(A>C) (rs1057910) in Iraqi T2DM patients:

Data presented in Table7 summarize the genotype and allele frequencies of CYP2C9*3 (A>C) (rs1057910) among 120 individuals with T2DM. The genotypic distribution was as follows: 80% AA, 15.8% AC, and 4.17% CC. The corresponding allele frequencies were 87% for allele A and 12.08% for allele C. Statistical analysis revealed a highly significant association between this SNP and the trait under investigation ($p < 0.007$), suggesting a potential role of this genetic variant in modulating the studied phenotype. In the context of the genetic analysis previously discussed, the Table8 displays detailed of the provided results for a specific genotype within a sample of (120) individuals calculated according to the Hardy-Weinberg equilibrium and utilizes the Fischer exact test to assess the observed genotype frequencies against their expected counterparts. The observed genotype percentages show allele (A) in 87% and allele (C) in 12.08% of the population, which deviated from Hardy-Weinberg equilibrium expected calculations, with allele (A) at 77.3% and allele (C) at 1.5 %. Fischer exact test yield significant p-values of 0.0203 for comparisons as AC/CA genotype and observed vs expected frequencies for AA, AC and CC genotypes.

Study the association between CYP2C9*3 rs1057910A>C Genotype and side effect of glibenclamide use in Iraqi T2DM patients.

Results presented in Table9 demonstrated a statistically significant association between the CYP2C9*3

(rs1057910 A>C) genotype and the occurrence of allergic reactions in T2DM patients receiving glibenclamide therapy (χ^2 = 6.22, df = 2, p = 0.0446). To enhance visualization, these findings were illustrated using a heat map Figure7, in which the X-axis represents the allergic reaction status (0 = No, 1 = Yes), while the Y-axis denotes the genotypes (AA, AC, CC). The color intensity in the heat map corresponds to the frequency distribution of cases across genotypes and reaction status. In contrast, no statistically significant associations were observed between CYP2C9*3 (rs1057910) genotypes and other reported adverse effects, including hypoglycemia, cardiovascular events (CVD), nausea, heartburn, or weight gain (p > 0.05).

Impact of CYP2C9*3 rs1057910 A>C genotype mutation on glibenclamide response with/without gender stratification in patients with T2DM:

This study investigated the influence of CYP2C9*3 (rs1057910 A>C) genotype and patient gender on therapeutic response to glibenclamide among individuals with T2DM. Patients were categorized into responders and non-responders based on glycemic control parameters, primarily HbA1c levels, and subsequently stratified by gender (female and male).

Among responders, 12% carried the wild-type genotype (AA) and 5% carried the mutant genotype (AC/CC), whereas among non-responders, 82% were wild-type and 21% were mutant. The proportion of non-responders with the wild genotype was significantly higher than that of responders with the wild genotype, as detailed in Table10.

Gender-based analysis further revealed that among responders, 8% of females and 4% of males carried the wild genotype, while 3% of females and 2% of males carried the mutant genotype. In contrast, among non-responders, 37% of females and 45% of males carried the wild genotype, while 10% of females and 11% of males carried the mutant genotype. Statistically, the proportion of non-responders with the wild genotype was significantly higher than that of responders with the mutant genotype, as shown in Table11. However, no statistically significant difference was observed within the male and female subgroups (p > 0.05).

Correlation between CYP2C9*3 rs1057910 gene variations and glycemic parameters in T2DM patients undergoing glibenclamide treatment.

Data presented in Table12 illustrate the association between CYP2C9*3 (rs1057910) genotypes (wild-type AA vs. mutant AC/CC) and key glycemic control parameters, including fasting blood glucose (FBG), HbA1c, HOMA-IR, HOMA-B, and insulin concentration, to assess the impact of genetic variation on glibenclamide response. Among patients carrying the mutant genotype (AC/CC), HOMA-B levels demonstrated a statistically significant negative correlation with FBG (p = 0.0453, Spearman's r = −0.40) and HbA1c (p = 0.0044, r = −0.55). Additionally, HOMA-IR showed a strong positive correlation with FBG (p = 0.0007, r = 0.63). FBG levels were also significantly correlated with HbA1c (p = 0.0425, r = 0.41). In the wild-type genotype (AA) group, HOMA-B exhibited significant correlations with HOMA-IR (p = 0.0122, r = 0.26), FBG (p = 0.0001, r = −0.55), and HbA1c (p = 0.0001, r = −0.63). HOMA-IR showed a strong positive correlation with FBG (p = 0.0001, r = 0.60). Similarly, FBG was significantly correlated with HbA1c (p = 0.0001, r = 0.64). These findings are visually summarized in the corresponding heatmap figure, where color intensity reflects both the strength and direction of the correlations: red/orange indicates positive correlations (variables increase together), blue indicates negative correlations (one variable decreases as the other increases), and white/neutral reflects weak or no correlation.

Genotype and allele frequency analysis of CYP2C9*2(C>T) (rs1799853) in Iraqi T2DM patients.

The provided data in the Table13 presents findings of the genotype and allele frequencies of the CYP2C9*2(C>T) (rs1799853) from (120) individuals. Analysis of genotypic distribution reveals 70% CC, 22.5 % CT and 7.50% TT genotypes. The major allele frequencies indicated 81.25% for allele (C) and 18.75 % for allele (T). Significant testing with a P-value=0.0003, indicating that CYPEC9 SNP is strongly linked to the trait under investigation.

Hardy-Weinberg equation analysis of CYP2C9*2(C>T) (rs rs1799853) in Iraqi T2DM patients.

Table14 represents findings of the CYP2C9*2 C>T genetic analyses, indicating genotype frequencies, Hardy-Weinberg equilibrium, Fischer exact test results and corresponding P-values. Fischer exact test has employed to compare expected to observed genotype frequencies, which reveals highly significant differences for CC, CT and TT genotypes alongside with P<0.00014.

Study the association between CYP2C9*2 rs1799853 C>T Genotype and side effect of glibenclamide use in Iraqi T2DM patients.

The results presented in Table15 demonstrated a highly significant association between the CYP2C9*2 rs1799853 C>T genotype and the occurrence of allergic reactions among patients with type 2 diabetes mellitus (T2DM) treated with glibenclamide (P-value = 0.0001). In contrast, no statistically significant associations were observed between the CYP2C9*2 rs1799853 C>T genotype and other adverse effects, including hypoglycemia, cardiovascular disease, nausea, heartburn, or weight gain (P-value > 0.05). These results suggest that the association of this genetic variant may be specific to allergic reactions rather than other glibenclamide-related side effects.

Impact of CYP2C9 *2 rs1799853 C>T genotype mutation on glibenclamide response with/without gender stratification in patients with T2DM:

The primary hypothesis of this study was to determine whether genetic variations in the CYP2C9 gene influence therapeutic response to glibenclamide in patients with type 2 diabetes mellitus (T2DM), potentially contributing to either treatment responsiveness or non-responsiveness. A total of 120 T2DM patients were genotyped for CYP2C9*2 rs1799853 C>T and subsequently categorized as responders or non-responders based on their plasma HbA1c levels. Among responders, 19% carried the wild-type genotype (CC) and 4% carried the mutant genotype (CT/TT), whereas among non-responders, 65% carried the wild-type genotype and 32% carried the mutant genotype. The proportion of non-responders with the wild-type genotype was significantly higher compared to responders with the same genotype, as detailed in Table16.

To further investigate potential gender-specific effects, patients were stratified by gender. Among female responders, 15% were wild-type and 1% were mutant, whereas among male responders, 4% were wild-type and 3% were mutant. In contrast, among female non-responders, 27% were wild-type and 15% were mutant, while among male non-responders, 38% were wild-type and 17% were mutant. Statistical analysis revealed that, within the female subgroup, the proportion of non-responders carrying the wild-type genotype was significantly higher than those carrying the mutant genotype ($p = 0.0452$), indicating a strong association between CYP2C9 rs1799853 C>T polymorphism and treatment response in females. However, no significant difference was observed within the male subgroup ($p = 0.671$), suggesting a possible gender-specific genetic influence on glibenclamide responsiveness, predominantly in females Table17.

Correlation between CYP2C9*2 rs1799853 gene variations C<T and glycemic parameters in T2DM patients undergoing glibenclamide treatment.

The results presented in Table18 illustrate the association between CYP2C9*2 rs1799853 genotypes (wild-type vs. mutant) and glycemic control parameters, including fasting blood glucose (FBG), HbA1c, HOMA-IR, HOMA-B, and plasma insulin concentration, to assess the impact of genetic variation on patient response to glibenclamide therapy. Among patients with the wild-type genotype (CC), HOMA-B showed a statistically significant correlation with HOMA-IR ($p = 0.0017$, $r = 0.338$), FBG ($p = 0.0001$, $r = –0.519$), and HbA1c ($p = 0.0001$, $r = –0.612$). Additionally, HOMA-IR exhibited a significant positive correlation with FBG ($p = 0.0001$, $r = 0.55$), while FBG demonstrated a strong positive correlation with HbA1c ($p = 0.0001$, $r = 0.628$).

In patients carrying the mutant genotypes (CT/TT), HOMA-B was significantly correlated with FBG ($p = 0.0004$, $r = –0.577$) and HbA1c ($p = 0.0001$, $r = –0.609$). Similarly, HOMA-IR showed a strong positive correlation with FBG ($p = 0.0001$, $r = 0.703$), and FBG was positively correlated with HbA1c ($p = 0.0005$, $r = 0.549$). These findings were visualized using a heat-map, in which the color intensity represents the strength and direction of the correlations: red/orange indicates a positive correlation (both variables increase simultaneously), blue indicates a negative correlation (as one variable increases, the other decreases), and white/neutral indicates no or a weak correlation.

DISCUSSION

The results presented in Table3 revealed a statistically significant difference in key glycemic parameters—including fasting blood glucose (FBG), glycated hemoglobin (HbA1c), HOMA-IR, and HOMA-B—between healthy controls and patients with type 2 diabetes mellitus (T2DM). These findings are consistent with the dual-pathophysiology of T2DM, characterized by the coexistence of insulin resistance and progressive β-cell dysfunction. Our results are in agreement with the study by Gandhi et al. (2017), which reported that routinely measured parameters such as FBG, HbA1c, and fasting serum insulin (FSI), along with calculated indices such as HOMA-IR and HOMA-B, were significantly altered in patients with T2DM. In the present study, plasma insulin concentrations did not differ significantly between T2DM patients and healthy controls. This finding may be attributable to the mechanism of action of glibenclamide, which promotes insulin secretion by inhibiting ATP-sensitive K^+ channels, leading to pancreatic β-cell depolarization and insulin release (Khalili and others, 2023). Additionally, significant gender-based differences in glycemic control parameters were observed, potentially reflecting variations in body mass index (BMI), hormonal profiles, and β-cell function between males and females. This observation is consistent with a study conducted at Debre Berhan Public Hospitals, which demonstrated notable sex-related disparities in glycemic control among T2DM patients (Fadhil and others, 2011). Figure (1) illustrates markedly higher FBG and HbA1c values among T2DM patients compared with healthy controls ($p < 0.0001$), confirming chronic hyperglycemia and suboptimal metabolic control. Elevated HbA1c values reflect prolonged glycemic exposure over 2–3 months and are strongly associated with microvascular complications. Although insulin levels were comparable between the two groups, the HOMA-IR index was significantly increased in T2DM patients, indicating substantial insulin resistance. Conversely, HOMA-B values were significantly reduced, consistent with impaired β-cell function (Cobelli and others, 2024). Collectively, these findings support the dual-defect model of T2DM, where insulin resistance and β-cell failure act synergistically to drive hyperglycemia. Recent studies have highlighted that elevated HOMA-IR predicts future cardiovascular events and kidney disease progression, whereas lower HOMA-B is associated with earlier initiation of insulin therapy. These results underscore the clinical relevance of early therapeutic strategies aimed at reducing insulin resistance and preserving β-cell function, such as the use of GLP-1 receptor agonists and SGLT2 inhibitors.

The results presented in Table4 revealed statistically significant differences in key lipid profile parameters—triglycerides (TG), high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein

cholesterol (LDL-C)—between healthy controls and patients with type 2 diabetes mellitus (T2DM). TG levels were significantly elevated in the diabetic group, whereas HDL-C and LDL-C levels were significantly lower compared with healthy controls. These findings are consistent with previous reports demonstrating elevated TG and reduced HDL-C as hallmarks of diabetic dyslipidemia. Earlier studies have also reported a strong association between HbA1c and lipid parameters, highlighting the critical importance of glycemic control in the management of dyslipidemia. Specifically, poorly controlled diabetes has been linked to a pronounced rise in TG levels and a concomitant reduction in HDL-C. (Gupta and Gupta, 2023)

In our study, a notable reduction in LDL-C levels was observed in the T2DM group compared with the control group, which may be attributable to the long-term effect of glibenclamide therapy on lipid metabolism. This pattern collectively represents the classical “diabetic dyslipidemia” phenotype, characterized by hypertriglyceridemia, low HDL-C, and a predominance of small, dense LDL particles—an atherogenic lipid profile strongly associated with accelerated atherosclerosis and increased cardiovascular risk (Chen and others, 2024). Gender-based analysis revealed significant differences in HDL-C levels between male and female patients with T2DM. These differences are consistent with the well-established influence of sex hormones on lipid metabolism. Estrogen reduces hepatic lipase activity, resulting in higher HDL-C levels, whereas testosterone increases hepatic lipase activity, leading to lower HDL-C concentrations. Consequently, women tend to exhibit higher HDL-C levels than men(Gorini et al., 2025). Figure (2) illustrates the characteristic pattern of diabetic dyslipidemia in T2DM patients: significantly elevated TG and LDL-C, along with reduced HDL-C, compared to healthy controls. Total cholesterol levels showed minimal differences between groups. TG levels were markedly elevated in both male and female patients ($p < 0.0001$), while HDL-C levels were significantly reduced ($p < 0.0001$), indicating impaired reverse cholesterol transport. LDL-C was significantly higher in male patients ($p = 0.0078$), further contributing to an atherogenic lipid profile. This combination of dysglycemia (elevated FBG, HbA1c, and insulin resistance) and atherogenic dyslipidemia represents a major driver of microvascular and macrovascular complications in T2DM. Recent evidence confirms that even in patients with good glycemic control, dyslipidemia remains a major determinant of cardiovascular morbidity and mortality. The 2024 ADA guidelines strongly recommend statin therapy for nearly all adults with T2DM, with the addition of ezetimibe or PCSK9 inhibitors in patients at very high ASCVD risk (Gupta and Gupta, 2023). These findings underscore the importance of comprehensive management strategies targeting not only glycemia but also weight control and lipid optimization to reduce long-term cardiovascular complications No statistically significant difference was observed in aspartate aminotransferase (AST) levels between T2DM patients and healthy controls. However, alanine aminotransferase (ALT) levels were significantly higher in the T2DM group. Elevated ALT, even within the upper normal range, is recognized as a predictor of T2DM and a biomarker for metabolic dysfunction–associated steatosis liver disease (MASLD; formerly NAFLD). MASLD shares a strong pathophysiological link with T2DM through mechanisms including hepatic insulin resistance, ectopic fat accumulation, and oxidative stress. This finding is consistent with recent guidance from the American Association for the Study of Liver Diseases (AASLD, 2023), which emphasizes the importance of early recognition and screening for MASLD in patients with T2DM as part of comprehensive diabetes care. Moreover, a significant gender-based difference was observed, with males exhibiting higher ALT levels than females. Although the underlying cause is not fully understood, this disparity may relate to differences in muscle mass, sex hormone profiles, and hepatic enzyme activity between genders(Rinella and others, 2023). Regarding to Renal function analysis, summarized in Table.6, demonstrated a significant difference in serum creatinine levels between T2DM patients and healthy controls, with elevated creatinine levels observed among T2DM patients. Creatinine remains a sensitive and widely used marker of early diabetic kidney disease (DKD), as rising creatinine levels often reflect declining glomerular filtration rate (GFR) before clinical symptoms of uremia appear. These results are in agreement with the KDIGO 2022 guidelines, which recommend routine monitoring of serum creatinine and calculation of estimated GFR (eGFR) as primary tools for the early

detection and management of DKD in diabetic populations. In contrast, serum urea levels showed no significant difference between T2DM patients and healthy controls. Interestingly, higher urea levels were noted among healthy females compared with T2DM patients, which could be attributed to transient factors such as dehydration, high-protein diets, and intense physical activity (Wahyuni et al., 2024).Collectively, these findings highlight the clinical importance of including liver and kidney function tests in the routine monitoring of T2DM patients. Elevated ALT may serve as an early warning marker for MASLD, while rising creatinine levels can indicate early renal impairment. Together, these parameters support the need for integrated metabolic, hepatic, and renal surveillance in diabetes management.

In the present study, we specifically investigated the association of CYP2C9*3 (rs1057910) A>C gene polymorphism with type 2 diabetes mellitus (T2DM). Table .7 summarizes the genotypic and allelic frequencies obtained from 120 T2DM patients. Genotypic distribution analysis revealed that 80% of individuals carried the AA genotype, 15.8% carried the AC genotype, and 4.17% carried the CC genotype. The corresponding allele frequencies were 87% for allele A and 12.08% for allele C (Nieh and Roman, 2025). The Hardy–Weinberg equilibrium (HWE) is a cornerstone in population genetics, stating that allele and genotype frequencies in a population remain constant across generations in the absence of evolutionary forces such as selection, migration, or genetic drift . HWE analysis provides an important framework for evaluating whether a given polymorphism follows expected Mendelian inheritance patterns. Comparison of the observed genotype frequencies with those expected under HWE revealed a statistically significant deviation for all genotypes. Specifically, the observed frequency of the AA genotype (80%) was higher than the expected frequency (77.3%), whereas the observed frequency of AC (15.8%) was lower than expected (21.3%). The CC genotype frequency (4.17%) was also significantly higher than its expected value (1.5%). Fisher's exact test confirmed a significant departure from HWE ($p = 0.0302$). These deviations from HWE suggest that one or more evolutionary or clinical factors may be influencing the distribution of CYP2C9*3 (rs1057910) genotypes within this T2DM cohort. Possible explanations include population stratification, non-random mating, or selection pressure due to differential drug response or disease susceptibility (Chen, 2010). Collectively, these findings underscore the potential contribution of CYP2C9*3 (rs1057910) A>C polymorphism to T2DM pathogenesis and variability in treatment response. Further large-scale, multi-ethnic studies integrating pharmacogenomic and functional analyses are warranted to elucidate the underlying mechanisms and to inform precision medicine approaches for optimizing glibenclamide therapy outcomes in diabetic populations.

The influence of CYP2C9*3 (rs1057910) single nucleotide polymorphism (SNP) on glibenclamide response in T2DM patients was evaluated, and the results are summarized in Table10 Among patients carrying the wild-type allele, 82% were classified as non-responders, compared with 21% in the mutant allele group. Although this trend suggests a lower frequency of non-response among carriers of the variant allele, the difference did not reach statistical significance. The odds ratio (OR) was found to be < 1, indicating a potential association between the C allele and reduced glibenclamide responsiveness; however, this association was weak and statistically non-significant. These findings may be partly explained by the polygenic nature of sulfonylurea response, where other pharmacogenes—such as ABCC8, KCNJ11, and CYP2C19—play an additional role in determining drug efficacy. Moreover, the relatively small number of AC/CC carriers in this cohort may have limited the statistical power to detect significant associations. When the data were stratified by gender, no significant correlation was observed between glibenclamide responsiveness and CYP2C9*3 genotype in either male or female patients. Although OR values below one continued to suggest lower sensitivity among variant carriers, the corresponding p-values confirmed the lack of statistical significance. It is noteworthy that CYP2C9*3 is generally associated with a reduction in enzymatic activity. Nevertheless, compensatory factors—such as clinical dose titration by treating physicians or alternative drug-metabolizing pathways—may mitigate the clinical impact of this polymorphism. The absence of a significant gender effect further suggests that sex hormones do not exert

a strong modulatory influence on CYP2C9*3 activity, unlike what has been observed with CYP2C9*2(Suzuki et al., 2006).

The results presented in Table 12 summarize the correlations between glycemic control parameters in T2DM patients receiving glibenclamide therapy, stratified by CYP2C9*3 (rs1057910) genotype , In patients carrying the mutant allele, HOMA-B demonstrated a statistically significant negative correlation with both FBG ($r = –0.40$, $p = 0.0453$) and HbA1c ($r = –0.55$, $p = 0.0044$), indicating that higher β-cell function is associated with lower fasting and long-term glucose levels. No significant correlations were observed between HOMA-B and either HOMA-IR or BMI. HOMA-IR showed a strong positive correlation with FBG ($r = 0.63$, $p = 0.0007$), suggesting that greater insulin resistance is associated with higher fasting glucose. However, HOMA-IR was not significantly correlated with HbA1c or BMI. FBG exhibited a moderate positive correlation with HbA1c ($r = 0.41$, $p = 0.0425$), reflecting the expected alignment between short-term and long-term glycemic measures. BMI was not significantly correlated with any glycemic parameter. These findings suggest that, in carriers of the mutant allele, β-cell function and insulin resistance operate in a less coordinated manner, potentially reflecting a disruption of normal glucose–insulin homeostasis associated with CYP2C9*3 polymorphisms. In patients with the wild-type genotype, HOMA-B showed a weak-to-moderate positive correlation with HOMA-IR ($r = 0.26$, $p = 0.0122$), indicating a modest association between β-cell function and insulin resistance. Importantly, HOMA-B exhibited a strong negative correlation with FBG ($r = –0.55$, $p = 0.0001$) and HbA1c ($r = –0.63$, $p = 0.0001$), suggesting that preserved β-cell function is strongly linked to lower fasting and long-term glucose exposure. No significant correlations were observed between HOMA-B and BMI. Similarly, HOMA-IR demonstrated a strong positive correlation with FBG ($r = 0.60$, $p = 0.0001$), but not with HbA1c or BMI. FBG correlated strongly and positively with HbA1c, confirming that fasting glucose is a reliable predictor of chronic glycemic control. The expected physiological relationships—namely, the inverse association between β-cell function and glucose levels, the positive association between insulin resistance and glucose, and the strong alignment of FBG with HbA1c—were preserved in both genotype groups. However, these correlations were generally stronger and more consistent in the wild-type group. The mutant group exhibited fewer statistically significant correlations overall, suggesting that CYP2C9*3 variants may partially disrupt the normal interplay between insulin secretion, insulin resistance, and glycemic control.Importantly, BMI was largely unrelated to other glycemic parameters in both groups, indicating that body mass alone does not explain the observed differences in glucose–insulin dynamics in this cohort. This finding underscores the importance of considering genetic factors, beyond anthropometric measures, when evaluating variability in treatment response to sulfonylureas.

In the present study, three genotypes of CYP2C9*2 (rs1799853 C>T) were identified among Iraqi patients with T2DM: CC (70%), CT (22.5%), and TT (7.5%). The corresponding allele frequencies were 81.25% for allele C and 18.75% for allele T. When evaluated under Hardy–Weinberg equilibrium (HWE), the expected genotype frequencies were CC (65.62%), CT (32.50%), and TT (1.88%). The observed distribution showed a statistically significant deviation from HWE expectations ($p = 0.00014$), suggesting a non-random distribution of genotypes within this patient cohort. These deviations may reflect population-specific factors, including genetic structure, ethnic background, environmental exposures, sample size, and analytical methodology. Such findings are of clinical relevance because CYP2C9*2 is a reduced-function allele associated with slower metabolism of sulfonylureas such as glibenclamide . Carriers of the T allele may be at higher risk of prolonged drug exposure, hypoglycemia, and altered therapeutic response. Therefore, these results support the potential utility of pharmacogenetic testing to guide individualized dosing and improve treatment safety and efficacy in T2DM patients of Iraqi descent (Saberi et al., 2020).

The responsiveness to glibenclamide therapy was assessed and is summarized in Table16 Among patients carrying the wild-type allele (CC), 65% were classified as non-responders, whereas only 19% were

responders. In the mutant allele group (CT/TT), 32% were non-responders and 4% were responders. Although these results suggest a numerically higher proportion of non-responders in the wild-type group, the overall analysis revealed no statistically significant association between CYP2C9*2 (rs1799853) genotype and glibenclamide responsiveness. This finding indicates that while CYP2C9*2 may exert a minor pharmacokinetic effect—given its known partial reduction in enzymatic activity—this effect appears insufficient to meaningfully alter the clinical response to glibenclamide in this cohort. Additional confounding factors such as dietary habits, medication adherence, and polypharmacy may have masked subtle genotype–phenotype associations (Rehman and others, 2023). To further explore potential gender-specific effects, glibenclamide responsiveness was analyzed by gender Table.17 Interestingly, a statistically significant association was observed between CYP2C9*2 genotype and treatment response in females ($p = 0.0452$). In this subgroup, the CC genotype was strongly associated with better therapeutic response, suggesting a sex-specific pharmacogenomic effect. By contrast, no significant association was detected in males, and the odds ratio even trended below 1, indicating a weaker response among wild-type carriers. These findings may be explained by gene-by-sex interactions, wherein the phenotypic effect of a genetic variant differs between males and females. Possible mechanisms include the modulatory influence of sex hormones on hepatic gene expression, sex-specific regulation of CYP2C9 transcription, and pharmacokinetic differences due to variations in fat distribution and drug metabolism between genders. For example, estrogen has been reported to upregulate CYP2C9 expression, potentially enhancing sulfonylurea metabolism and influencing drug responsiveness in females. It is also important to note that the number of male CC carriers in the responder group was relatively small ($n = 4$), which may have limited the statistical power to detect significant associations. Thus, while the observed female-specific association is intriguing, the findings require confirmation in larger, adequately powered cohorts to establish their clinical significance (Kautzky-Willer et al., 2023) .

Overall, these results suggest that female T2DM patients carrying the CC genotype of CYP2C9*2 may respond more favorably to glibenclamide therapy and could benefit from individualized dosing strategies. Integrating pharmacogenetic testing into routine clinical practice—particularly for female patients—may help optimize sulfonylurea therapy, minimize treatment failure, and reduce the risk of hypoglycemia. Further large-scale, prospective studies are warranted to validate these findings and clarify the role of gender-specific pharmacogenomics in diabetes management.

Table .18 examines the relationship between metabolic parameters—including HOMA-B (β-cell function), insulin resistance (HOMA-IR), fasting blood glucose (FBG), HbA1c, and BMI—in T2DM patients receiving glibenclamide therapy, stratified by CYP2C9*2 (rs1799853) genotypes (wild-type CC vs. mutant CT/TT). Given that CYP2C9 polymorphisms influence sulfonylurea metabolism, analyzing these correlations provides insight into how β-cell function and insulin resistance contribute to differential drug responses.In the wild-type group, HOMA-B exhibited a significant positive correlation with HOMA-IR ($r = 0.338$, $p = 0.0017$), suggesting that as β-cell function increases, insulin resistance also rises—likely reflecting compensatory hyperinsulinemia in response to peripheral insulin resistance. HOMA-B showed a strong negative correlation with FBG ($r = –0.519$, $p = 0.0001$) and HbA1c ($r = –0.612$, $p = 0.0001$), indicating that better β-cell function is associated with improved glycemic control and lower long-term glycemic burden.

HOMA-IR demonstrated a strong positive correlation with FBG ($r = 0.55$, $p = 0.0001$), consistent with the pathophysiology of T2DM where increasing insulin resistance contributes to hyperglycemia. Similarly, FBG was strongly positively correlated with HbA1c ($r = 0.628$, $p = 0.0001$), confirming that fasting glucose levels are a reliable indicator of chronic glycemic exposure. BMI, however, showed no significant correlation with HOMA-B, HOMA-IR, FBG, or HbA1c, suggesting that body mass is not a major determinant of glycemic parameters in this subgroup. In contrast, patients carrying the mutant allele

exhibited a weaker and statistically non-significant association between HOMA-B and HOMA-IR ($r = 0.115$, $p = 0.5054$), suggesting diminished compensatory β-cell activity in the presence of insulin resistance. HOMA-B remained significantly and negatively correlated with FBG ($r = –0.557$, $p = 0.0004$), indicating that preserved β-cell function still contributes to lowering fasting glucose, but its compensatory effect appears less robust compared with the wild type.

HOMA-IR showed an even stronger positive correlation with FBG ($r = 0.703$, $p = 0.0001$), suggesting that insulin resistance is a more dominant driver of hyperglycemia in mutant carriers. FBG retained a positive correlation with HbA1c ($r = 0.549$, $p = 0.0005$), though slightly weaker than in wild-type individuals. BMI again displayed no significant associations with any of the metabolic parameters. These findings suggest that carriers of the CYP2C9*2 variant have impaired β-cell compensation for insulin resistance, resulting in a stronger influence of HOMA-IR on hyperglycemia and more pronounced glycemic dysregulation. In contrast, wild-type carriers demonstrate a more coordinated glucose–insulin relationship, allowing for better compensation against rising insulin resistance (Gandhi and others, 2017).

Clinically, these results imply that CYP2C9*2 variant carriers may require closer glycemic monitoring and potentially adjusted therapeutic strategies, such as individualized sulfonylurea dosing or earlier initiation of combination therapy with insulin sensitizers. These finding supports that CYP2C9 genetic polymorphisms contribute to interindividual differences in both glycemic control and therapeutic response to sulfonylureas, underscoring the relevance of pharmacogenetic testing in guiding precision medicine approaches for T2DM.

This schematic illustrates the hypothesized influence of CYP2C9 polymorphisms (rs1057910 A>C, rs1799853 C>T) on glibenclamide metabolism, therapeutic response, and adverse effects in T2DM patients. Genetic variation can alter drug clearance, leading to variability in glycemic control, where some patients achieve good response (HbA1c < 7%) and others remain non-responsive (HbA1c ≥ 7%). The model also highlights the observed association between the wild-type genotype and increased risk of allergic reactions, as well as a gender effect, with females showing higher susceptibility to both allergic reactions and differences in drug response. Collectively, the diagram emphasizes the multifactorial nature of sulfonylurea response, suggesting that integrating genetic and sex-related factors into clinical decision-making could improve personalization of therapy and optimize outcomes.

CONCLUSION

The present study suggests that CYP2C9 gene polymorphisms (rs1057910 A>C and rs1799853 C>T) may alter glibenclamide metabolism, leading to interindividual variability in therapeutic response among patients with Type 2 Diabetes Mellitus. While no significant association was observed between these polymorphisms and glycemic response, a notable gender-related effect was identified, indicating that female patients exhibited higher sensitivity and a greater incidence of allergic reactions, particularly among those carrying the wild-type genotype. Overall, the findings emphasize that genetic background and gender differences contribute to the variability in glibenclamide efficacy and tolerability, highlighting the importance of integrating pharmacogenetic and demographic factors into personalized diabetes therapy.

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Figure legends:

Figure1: Allele specific PCR amplification for rs1057910 A<C showing a distinct band with a molecular size of 420 bp for samples. The size of amplification was ascertained by comparing it to a 100-1500 by DNA ladder, and the CYP2C9*3 rs1057910 was categorized into three genotypes: AA (wild considered as major), AC (mutant heterozygote) and CC (mutant homozygote).

Figure2: Allele specific PCR amplification for rs1799853C<T showing a distinct band with a molecular size of 420 bp for samples. The size of amplification was ascertained by comparing it to a 100-1500 by DNA ladder, and the CYP2C9*2 rs179953 was categorized into three genotypes: CC (wild considered as major), CT (mutant heterozygote) and TT (mutant homozygote).

Figure3: Comparison Glycemic Control, Insulin Resistance, And Beta-Cell Function Between T2DM Patients and Healthy Subjects., A) FBG: fasting blood glucose, B) HBAIC: hemoglobulin A1c, C) HOMA-IR, D) HOMA-B: Homeostasis Model Assessment of Beta-cell Function, E) Insulin. The data presented as mean±SD, P<0.05 considered significant.

Figure4: Comparison of Blood Lipid Profile Between T2DM Patients and Healthy Subjects. A) CHOL: Cholesterol, B) TG: triglyceride, C) HDL: high density lipoprotein, D) LDL: low density lipoprotein. Data presented as mean±SD, P<0.05 considered significant.

Figure5: Comparison of Liver Function Enzymes Between Patients with T2DM and Healthy Subjects. A) ALT:Alanine Aminotransferase, B) AST:Aspartate Aminotransferase. Data presented as mean±SD, P<0.05 considered significant.

Figure.6: Comparison of kidney Functions Indicators in Patients with T2DM and Healthy People. A) Urea and B) Creatinine. Data presented as mean±SD, P<0.05 considered significant. P<0.05 considered significant.

Figure7: A heatmap shows allergic reaction by CYP2C9*3 rs1057910 A>C Genotype. X-axis refers to the status of the patients experienced an allergic reaction (0 = No, 1 = Yes). The Y-axis shows the genotype (AA, AC, CC). The color intensity reflects the frequency of cases.

Geneti

Figure1

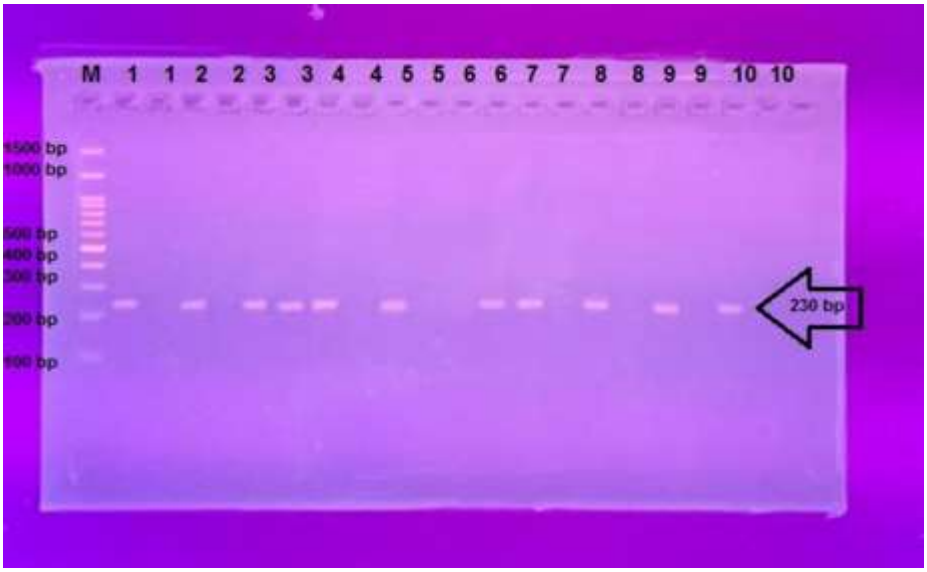

Figure2

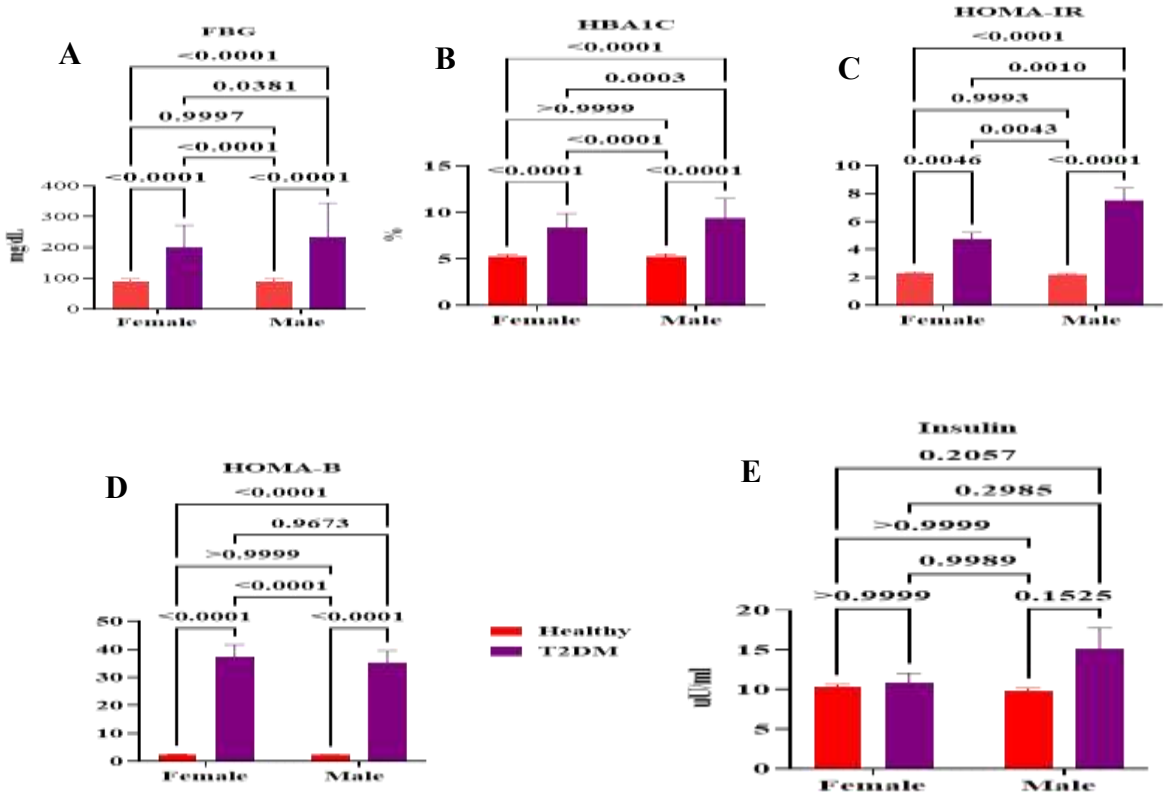

Figure3

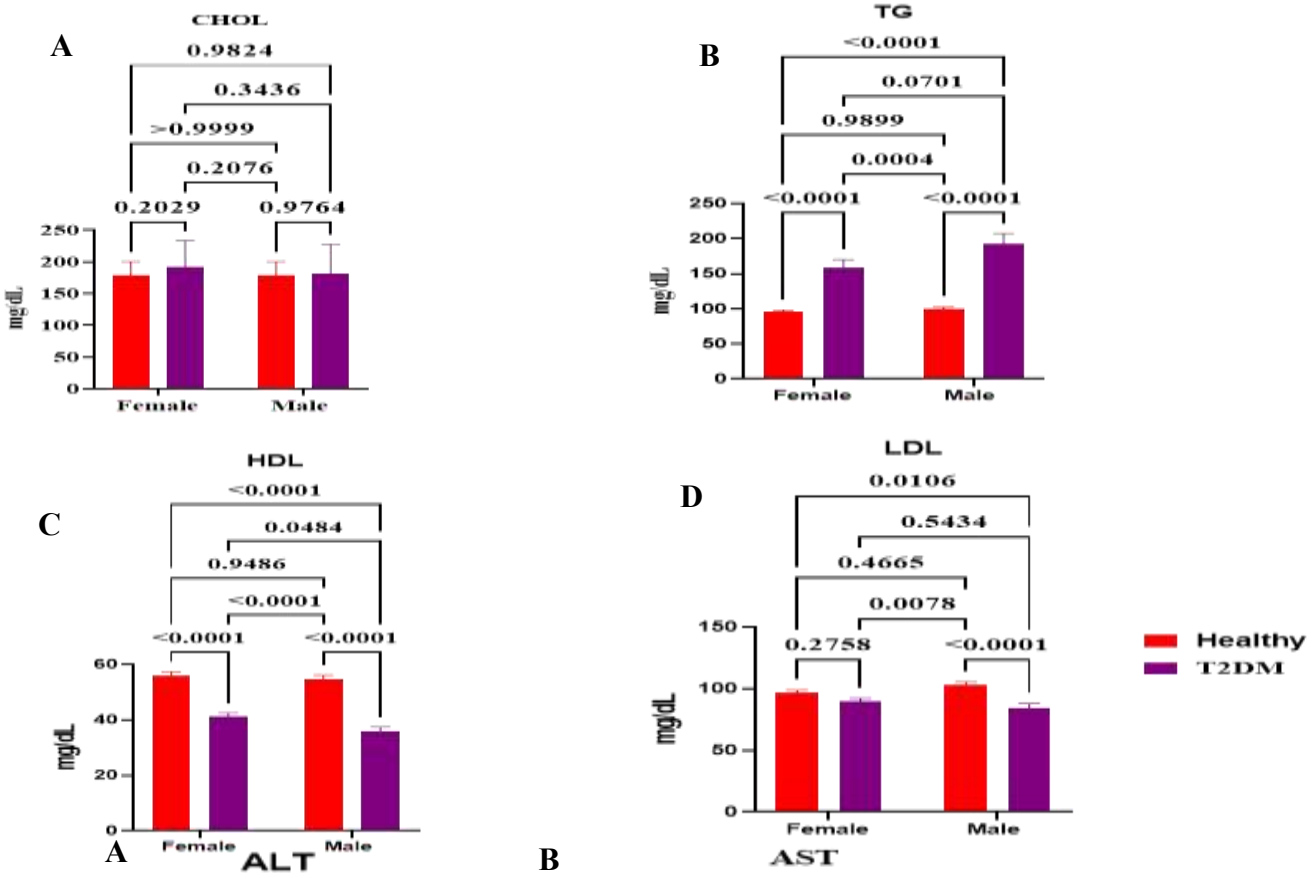

Figure4

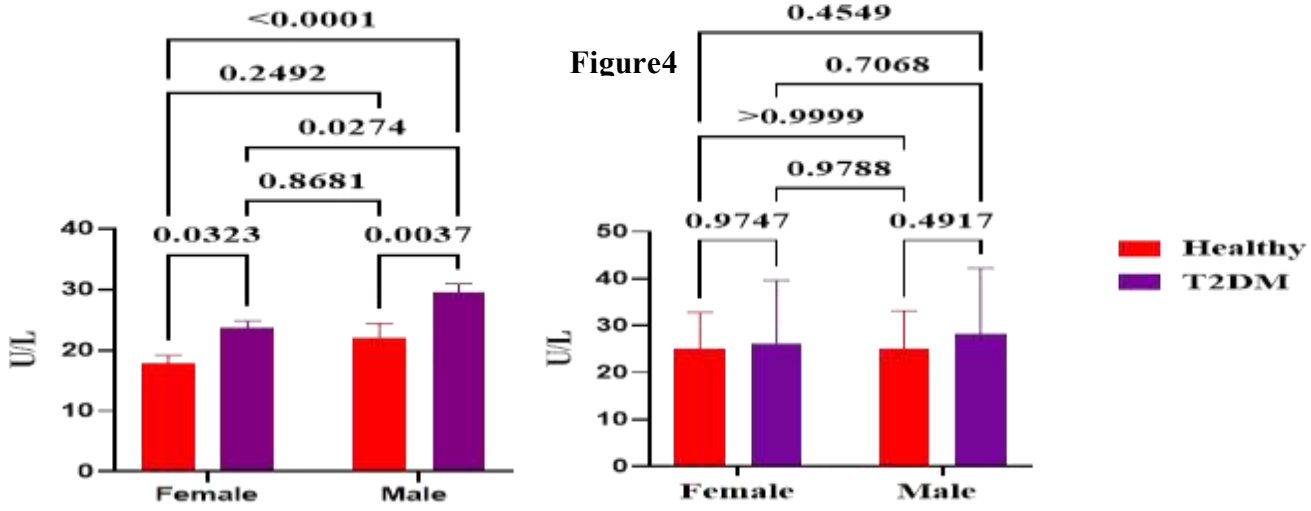

Figure5

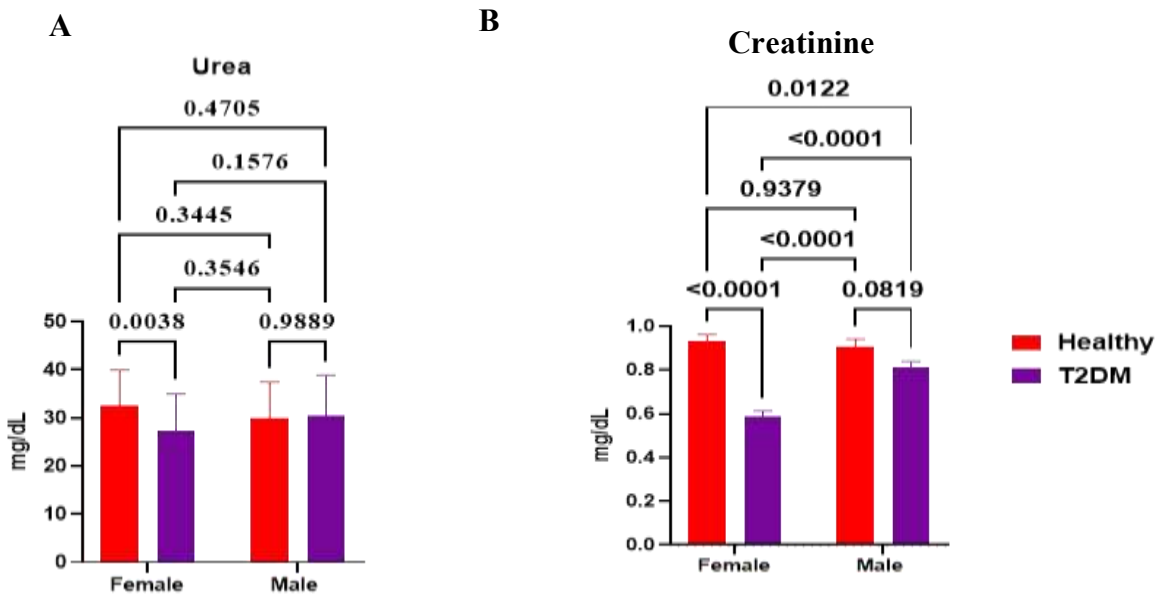

Figure6

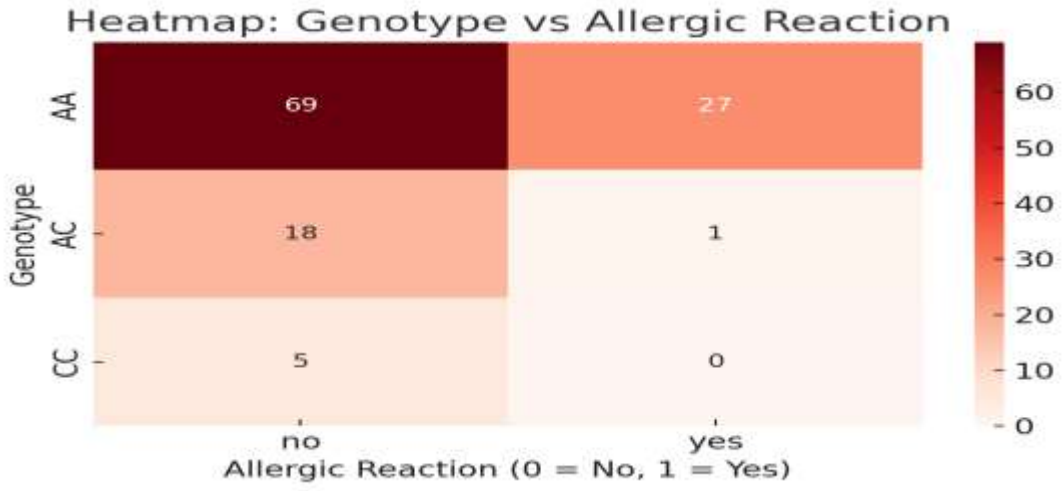

Figure7

Table1: Primer sequence of CYP2C9*2 (rs1799853)

Primer	Sequence (5′-3′)	Product size (bp)
Un-delete	ATGGGGAAGAGGAGCATTGAGGACC	230
Del-C	ATGGGGAAGAGGAGCATTGAGGACT	230
O-R	TTCCCTTGGCTCTCAGCTTCAAACCC	230

Table2: Primer sequence of CYP2C9*3 (rs1057910)

Primer	Sequence (5′-3′)	Product size (bp)
Un-delete	GGTGCACGAGGTCCAGAGATACA	420
Del-A	GGTGCACGAGGTCCAGAGATACC	420
O-R	CACCCTGCCAGAAATTCCAGCCCAA	420

Table3: Glycemic Control, Insulin Resistance, and Beta-Cell Function in T2DM patients

Healthy, n=100 mean±SD		**T2DM, n=120 mean±SD**	
FBG, mg/dl			
Female	**Male**	**Female**	**Male**
89.614±9.9190	90.909±9.143	200.217±73.084	234.512±109.904
Insulin, µU/ml			
10.35 ± 2.23	9.88 ± 2.09	10.87 ± 9.31	15.12 ± 20.94
HBA1C%			
5.223±0.235	5.241±0.298	8.365±1.539	9.396±2.094
HOMA-IR			
2.298±0.582	2.206±0.477	4.734±3.633	7.484±5.431
HOMA-B			
2.43 ± 0.57	2.30 ± 0.58	37.28 ± 33.34	35.20 ± 33.21
Data presented as mean±SD, P<0.05 considered significant, FBG: fasting blood glucose, HBAIC: hemoglobulin A1c, HOMA-B: Homeostasis Model Assessment of Beta-cell Function, HOMA-IR: Homeostasis Model Assessment of Insulin Resistance.			

Table4: Assessment of blood lipid profile of T2DM patients compared to healthy subjects

Healthy, n=100 mean±SD		**T2DM, n=120 mean±SD**	
Cholesterol, mg/dl			
Female	**Male**	**Female**	**Male**
179.05 ± 21.13	178.69 ± 21.66	192.28 ± 41.00	181.51 ± 46.08
Triglycerides, mg/dl			
95.54 ± 18.00	100.13 ± 18.89	159.16 ± 82.81	192.74 ± 113.40
HDL, mg/dl			

55.81 ± 11.25	54.59 ± 10.06	41.16 ± 10.21	35.83 ± 12.82
LDL, mg/dl			
96.90 ± 14.36	103.30 ± 14.31	89.38 ± 21.62	84.03 ± 30.69
Data presented as mean±SD, P<0.05 considered significant. TG: triglyceride, HDL: high density lipoprotein, LDL: low density lipoprotein			

Table5: Analysis of liver function enzymes in Patients with T2DM compared to healthy subjects

Healthy, n=100 mean±SD		**T2DM, n=120 mean±SD**	
ALT, U/L			
Female	**Male**	**Female**	**Male**
17.83 ± 9.51	22.04 ± 16.31	23.76 ± 7.85	29.58 ± 10.58
AST, U/L			
25.21 ± 7.64	25.24 ± 7.96	26.13 ± 13.55	28.37 ± 13.91
Data presented as mean±SD, P<0.05 considered significant. ALT:Alanine Aminotransferase, AST:Aspertate Aminotransferase.			

Table6: Measurement of kidney Functions Indicators in Patients with T2DM and Healthy People

Healthy, n=100 mean±SD		**T2DM, n=120 mean±SD**	
Urea, mg/dL			
Female	Male	Female	Male
32.51 ± 7.47	29.90 ± 7.59	27.36 ± 7.62	30.38 ± 8.47
Creatinine, mg/dL			
0.909 ± 0.22	0.91 ± 0.231	0.590 ± 0.174	0.81 ± 0.199
Data presented as mean±SD, P<0.05 considered significant.			

Table7: Genetic variation analysis of CYP2C9*3 rs1057910 A>C in T2DM patients (n=120)

Gene	Genotype	Group n=120	Frequency%	Allele		P value
				A	C	
*CYP2C9*3 rs1057910*	AA	96	80.00	0.879167	0.120833	<0.007
	AC	19	15.83			
	CC	5	4.17			
The statistic calculation by Chi-square statistic = 138.02, P value <0.05 considered significant, data shows as percentage.						

Table8: Hardy–Weinberg equilibrium for CYP2C9*3 rs1057910 A>C genotype in T2DM patients

Genotype	**Observed (n=120)**	**Observed %**	**Expected Count**	**Expected %**	**Alleles**	**HWE P-value**
AA	96	80.0	92.75	77.3	A	0.0203
AC	19	15.8	25.5	21.3	A/C	
CC	5	4.2	1.75	1.5	C	

Allele Freq.	—	—	—	—	A: 87.92% C: 12.08%	
The distribution significantly deviates from Hardy–Weinberg equilibrium (HWE) (P = 0.0203, p < 0.05)						

Table9: Study the association between side effects of Glibenclamide use and CYP2C9*3 rs1057910 A>C Genotype**:**

Table10: Association between *CYP2C9*3* rs1057910 A>C Genotype and Glibenclamide response across all patients, without gender stratification.

Genotype	**Responders**	**Non-Responders**	**Odds Ratio**	**P-value**
AA	12	82	0.614	0.524
AC/CC	5	21		
The data calculated using Fisher's Exact Test, P<0.05 is consider significant				

Table11: Association Between CYP2C9*3 rs1057910 A>C Genotype and Glibenclamide

Gender		**Responders (AA)**	**Non-Responders (AA)**	**Responders (AC/CC)**	**Non-Responders (AC/CC)**	**Odds Ratio**	**P-value**
Female		8	37	3	10	0.7207	0.696
Male		4	45	2	11	0.4888	0.596
	The data calculated using Fisher's Exact Test, P<0.05 is consider significant						

Responsiveness Stratified by Gender

Table12: Spearman correlation coefficients (r) and p-values for glycemic parameters with *CYP2C9*3* rs1057910 A>C Genotype

Side Effect	χ^2	df	P-value
CVD	11.44	32	0.9997
Allergic reaction	6.22	2	0.0446
G/I problems	0.52	2	0.7705
Weight gain	4.30	2	0.1165
Hypoglycemia	1.42	4	0.8410

Group based *CYP2C9*3* rs1057910 A>C Genotype	**Variable 1**	**Variable 2**	**Correlation Coefficient (r)**	**P-value**
Mutant	HOMA-B	IR	0.32	0.1217
Mutant	HOMA-B	FBG	-0.4	0.0453
Mutant	HOMA-B	HBAIC	-0.55	0.0044
Mutant	HOMA-B	BMI	-0.26	0.2164
Mutant	IR	FBG	0.63	0.0007
Mutant	IR	HBAIC	-0.04	0.8436
Mutant	IR	BMI	-0.11	0.6019
Mutant	Blood G	HBAIC	0.41	0.0425

Mutant	Blood G	BMI	0.06	0.7603
Mutant	HBAIC	BMI	-0.02	0.9403
Wild	HOMA-B	IR	0.26	0.0122
Wild	HOMA-B	Blood G	-0.55	0.0001
Wild	HOMA-B	HBAIC	-0.63	0.0001
Wild	HOMA-B	BMI	0.17	0.0984
Wild	IR	Blood G	0.6	0.0001
Wild	IR	HBAIC	0.17	0.1099
Wild	IR	BMI	-0.01	0.9439
Wild	Blood G	HBAIC	0.64	0.0001
Wild	Blood G	BMI	-0.13	0.2001
Wild	HBAIC	BMI	-0.08	0.4611
$P < 0.05 \rightarrow$ Statistically significant $P \geq 0.05 \rightarrow$ Not significant Wild (AA) Group: HOMA-B vs. HbA1c $\rightarrow p < 0.00001$ IR vs. HOMA-B $\rightarrow p = 0.012$ Blood Glucose vs. HbA1c $\rightarrow p < 0.00001$ Mutant (AC/CC) Group: HOMA-B vs. HbA1c $\rightarrow p = 0.004$ IR vs. Blood Glucose $\rightarrow p = 0.0007$ HOMA-B vs. Blood Glucose $\rightarrow p = 0.045$				

Table13: Genetic variation analysis of CYP2C9*2 rs1799853 C>T in T2DM patients (n=120)

Gene	**Genotype**	**Group n=120**	**Frequency%**	**Allele**		**P value**
				C	**T**	
*CYP2C9*3 rs1799853*	CC	84	70.00	0.8125	0.1875	0.0003
	CT	27	22.50			
	TT	9	7.50			
The statistic calculation by Chi-square statistic = 93.75, P value <0.05 considered significant, data shows as percentage.						

Table14: Hardy–Weinberg Equilibrium for CYP2C9*2 rs1799853 (C>T) genotype in T2DM patients

Genotype	**Observed (n=120)**	**Observed %**	**Expected Count**	**Expected %**	**Alleles**	**HWE P-value**
CC	84	70.00	78.75	65.62	C	0.00014
CT	27	22.50	39.00	32.50	C/T	
TT	9	7.50	2.25	1.88	T	
Allele Freq.	—	—	—	—	C:0.8125	
					T: 0.1875	
The distribution significantly deviates from Hardy–Weinberg equilibrium (HWE) (P = 0.00014, p < 0.05)						

Table16: Association between CYP2C9*2 rs1799853 C>T genotype and Glibenclamide response across all patients, without gender stratification.

Genotype	Responders	Non-Responders	Odds Ratio	P-value
CC	19	65	2.338	0.205
CT/TT	4	32		
The data calculated using Fisher's Exact Test, P<0.05 is consider significant				

Table17: Association between *CYP2C9*2 rs1799853* C>T genotype and Glibenclamide response across all patients, gender stratification.

Gender	Responders (CC)	Non-responders (CC)	Responders (CT/TT)	Non-responders (CT/TT)	Odds Ratio	P-value
Female	15	27	1	15	8.333	0.0452
Male	4	38	3	17	0.596	0.671
The data calculated using Fisher's Exact Test, P<0.05 is considered significant						

Table18: Spearman correlation coefficients (r) and p-values for glycemic parameters with *CYP2C9*2 rs1799853* C>T Genotype

Group Based *CYP2C9*2 rs1799853* C>T Genotype	Variable 1	Variable 2	Correlation Coefficient (r)	P-value
Wild	HOMA-B	IR	0.338	0.0017
Wild	HOMA-B	Blood G	-0.519	0.0001
Wild	HOMA-B	HBAIC	-0.612	0.0001
Wild	HOMA-B	BMI	0.022	0.8402
Wild	IR	Blood G	0.55	0.0001
Wild	IR	HBAIC	0.115	0.2986
Wild	IR	BMI	-0.052	0.6394
Wild	Blood G	HBAIC	0.628	0.0001
Wild	Blood G	BMI	-0.068	0.5359
Wild	HBAIC	BMI	-0.079	0.4764
Mutant	HOMA-B	IR	0.115	0.5054
Mutant	HOMA-B	Blood G	-0.557	0.0004
Mutant	HOMA-B	HBAIC	-0.609	0.0001
Mutant	HOMA-B	BMI	0.214	0.2175
Mutant	IR	Blood G	0.703	0.0001
Mutant	IR	HBAIC	0.204	0.2332
Mutant	IR	BMI	0.034	0.8473
Mutant	Blood G	HBAIC	0.549	0.0005
Mutant	Blood G	BMI	-0.232	0.1794
Mutant	HBAIC	BMI	-0.037	0.8317
P < 0.05 → Statistically significant				

P ≥ 0.05 → Not significant Wild (CC) Group HOMA-B vs. HbA1c → p = 0.0001 HOMA-B vs. Blood Glucose → p = 0.0001 IR vs. Blood Glucose → p = 0.0001 Blood Glucose vs. HbA1c → p = 0.0001 HOMA-B vs. IR → p = 0.0122 Mutant (CT/TT) Group HOMA-B vs. HbA1c → p = 0.0044 HOMA-B vs. Blood Glucose → p = 0.0453 IR vs. Blood Glucose → p = 0.0007 Blood Glucose vs. HbA1c → p = 0.0425