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Original Article

Dual Impact Analysis of GSTT1 Polymorphisms on the Metformin Response and Cardiovascular Risk: A Novel Integrated Approach in Type 2 Diabetes Mellitus Management

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ABSTRACT

Background: Although genetic variations in glutathione S-transferase (GST) have been linked to type 2 diabetes mellitus (T2DM), the relationships among GSTT1 polymorphisms, metformin effectiveness, and cardiovascular risk remain unclear. **Objective:** This study investigated the association between the GSTT1 null genotype and the metformin monotherapy response while simultaneously assessing its impact on atherosclerotic cardiovascular disease risk. **Methods**: This case-control study examined 84 T2DM patients aged 30-70 years, categorized into metformin therapy responders and nonresponders. This study employed multiplex PCR for GSTT1 genotyping and utilized the pooled cohort equation for cardiovascular risk assessment, offering a comprehensive analysis of both genetic and clinical parameters. **Results**: GSTT1 genetic variations were not significantly associated with metformin response (odds ratio [OR] 1.773, p=0.306) or cardiovascular risk (OR 1.086, 95% CI: 0.46-2.56, p=1.000). The analysis revealed distinct patterns in clinical parameters between the GSTT1 null and wild-type genotypes, particularly in terms of glycemic control indicators and lipid profiles, although these differences did not reach statistical significance. **Conclusion**: This study presents a novel approach by simultaneously examining both the therapeutic response and cardiovascular risk in relation to GSTT1 polymorphisms, distinguishing it from previous studies that typically focused on either aspect in isolation. These findings suggest that while genetic variations in GSTT1 may influence metabolic parameters, their direct impact on metformin effectiveness and cardiovascular risk may be more complex than previously thought.

KEYWORDS

GSTT1 polymorphism; metformin; cardiovascular risk; type 2 diabetes mellitus; pharmacogenetics

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INTRODUCTION

Diabetes is the last stage of a chronic and accelerating disorder resulting from insulin resistance, decreased pancreatic β-cell function, and elevated glucose levels. All patients with diabetes mellitus are likely to have insulin resistance [1]. Posttranslational modifications and gene mutations are thought to play a role in the disruption of cell signaling pathways that can lead to insulin resistance [1]. Several genes are thought to be involved in cellular pathways involved in glucose metabolism and storage. Changes in these genes can cause diabetes or are risk factors for diabetes. These genes include adiponectin [2], protein tyrosine phosphatase nonreceptor type 1 (PTPN1), hepatocyte nuclear factor 1 homeobox B (HNF-1β) [3,4], peroxisome proliferator activated receptor gamma (PPARG), and glutathione S-transferase (GST) [1].

Glutathione S-transferases (GSTs) are dimeric proteins that catalyze the conjugation reaction between glutathione and aromatic heterocyclic radicals and epoxides [5]. The GST enzyme consists of several subfamilies, including glutathione S-transferase M1 (GSTM1) and glutathione S-transferase T1 (GSTT1). Damage to GST enzymes is thought to result in inefficient detoxification, leading to genetic damage and increased risk of disease. The GSTM1- or GSTT1-null genotype is associated with various diseases [6]. Studies in Egypt have shown that the proportion of diabetes mellitus patients with the GSTM1 and GSTT1 null genotypes is significantly greater in T2DM patients. A T2DM study in a Chinese population revealed that GSTM1 positivity was greater than the GSTM1 null genotype and that the GSTT1 null genotype was greater than the GSTT1 positivity [7]. A study in T2DM patients in Iraq revealed that there were more GSTM1- and GSTT1-null genotypes than GSTM1- and GSTT1-positive genotypes [8]. A previous study in T2DM patients revealed that GSTM1 and GSTT1 positivity was greater than GSTM1 and GSTT1 null genotypes in Brazil [9]. T2DM patients who carry both genetic variations have a 3.17-fold increased risk compared with those with normal genotypes.

Measuring GST levels in T2DM patients is expected to help predict the occurrence of complications in patients with T2DM. In previous studies, a decrease in GST levels was observed in patients with T2DM, along with an increase in HbA1c. One study revealed a relationship between HbA1c and GST; in T2DM patients with high HbA1c, high GST levels decreased significantly. In patients with T2DM, the duration of time suffering from T2DM can also affect GST levels [8]. The longer a person has suffered from T2DM, the greater the decrease in GSH levels in the body [8]. GST levels were lower in T2DM patients with HbA1c >7 mg/dL than in T2DM patients with HbA1c <7 mg/dL. Therefore, the study concluded that there was a negative correlation between HbA1c and GST levels [10].

Excessive blood sugar levels can also increase indicators of oxidative stress, in which free radicals play important roles in the pathogenesis of DM complications. Oxidative stress can trigger T2DM by reducing insulin sensitivity and damaging pancreatic β-cells[10]. In patients with T2DM, oxidative stress is known to affect T2DM and lead to complications, such as heart disease, diabetes, stroke, and CVD. Oxidative stress is induced by an increase in free fatty acids (FFAs), which causes insulin resistance and pancreatic β-cell damage through the activation of stress-sensitive signaling pathways. Other oxidative stress pathways can occur and play a role in the pathogenesis of diabetes by triggering macromolecular damage. In addition, it functions as a signaling molecule to activate stress-sensitive pathways [9]. Therefore, it is associated with an increased risk of DM in GSTM1- and GSTT1-null genotypes because of the lack of protection against oxidative stress in cells [9].

In recent years, several studies have investigated the relationship between GSTM1/GSTT1 polymorphisms and T2DM risk; however, the results of these studies are not always concordant. Yalin [9] revealed that the GSTM1 polymorphism plays an important role in T2DM development, but the GSTT1 polymorphism was not significantly associated with T2DM development. In a study by [11], the results showed that high significance could not increase the association between the GSTM1 and GSTT1 genotypes in T2DM patients. Therefore, the effects of GSTM1 and GSTT1 polymorphisms on the development of T2DM are still unclear.

Metformin (1,1-dimethylbiguanide hydrochloride) was the first oral blood glucose-lowering treatment for type 2 diabetes [11]. The oral hypoglycemic drug metformin was used as the drug to be tested because one of the mechanisms of action of metformin is to increase the binding and number of insulin receptors. Metformin can alter its effectiveness when blood sugar levels are affected by GSTM1 and GSTT1. Metformin has cardiovascular benefits and is used to treat diabetes mellitus during pregnancy and early childhood. Pretreatment of diabetic patients with metformin was found to be associated with a reduction in myocardial infarction (MI) size compared with that of patients not treated with metformin [6].

Variations in the response to metformin, with controlled metformin therapy, can reduce the risk of macrovascular and microvascular abnormalities. Metformin reduces blood sugar levels by normalizing glycemic conditions and reducing the toxic effects of glucose on the pancreas to improve β-cell function [6]. Uncontrolled blood sugar levels and uncontrolled metformin therapy can lead to CVD complications such as coronary heart disease, cerebral vascular disease, and peripheral vascular disease. There are no clear studies on the variation analysis and effectiveness between GSTM1 and GSTT1 null genotypes with metformin monotherapy in diabetes mellitus patients and the prediction of genetic variation in increasing the risk of atherosclerotic cardiovascular disease in the next 10 years. Therefore, this study aimed to analyze GSTM1 and GSTT1 null genotypes as possible genetic factors associated with metformin monotherapy and CVD risk in T2DM patients.

MATERIALS AND METHODS

Study design, setting and population

This case–control study investigated patients with type 2 diabetes mellitus (T2DM) by examining risk factors influencing treatment effects [12]. The study population was derived from a previous study titled "The Effect of PRKAA2 Genetic Variation on the Effectiveness of Metformin Therapy in Type 2 Diabetes Mellitus Patients." A total of 130 patients with T2DM aged 30–70 years were recruited. The case group comprised nonresponders to metformin therapy, whereas the control group included metformin therapy responders.

Subject selection criteria

The study established comprehensive inclusion criteria for both the case and control groups. For the case group, eligible participants were type 2 diabetes mellitus patients aged between 30 and 70 years who exhibited complications and demonstrated nonresponsiveness to metformin therapy. These patients were required to have HbA1c levels ranging from 7% to 9% at the time of enrollment. Only patients newly diagnosed with T2DM who were receiving metformin therapy were considered. Additionally, participants were required to provide adequate nondegraded DNA samples for genetic analysis. Written informed consent was obtained from all participants prior to their enrollment in the study. The control group followed similar baseline criteria but specifically comprised patients who demonstrated a positive therapeutic response to metformin treatment.

To maintain data quality and analytical validity, the study implemented specific exclusion criteria. Participants were excluded if their DNA samples showed any signs of degradation during laboratory analysis, which could compromise the genetic testing results. Additionally, patients with incomplete medical records or missing essential clinical data were excluded to ensure a comprehensive analysis and reliable conclusions. The study also excluded cases in which the quantity of DNA obtained was insufficient to complete the required genetic analysis. These strict exclusion criteria were implemented to maintain the integrity of the research findings and to ensure reliable data interpretation.

Molecular analysis

DNA Extraction and PCR Analysis

Peripheral blood samples (10 mL) were collected, and DNA was extracted via the salting-out method. Multiplex PCR was performed via three primer pairs for GSTM1 and β-globin (control). The PCR mixture (1.05 mL) contained GoTaq Green Master Mix (including MgCl2, dNTPs, Taq DNA polymerase, and 10x PCR buffer), forward and reverse primers (84 μL each), 798 μL of sterile ddH_2O, and 1 µL of template DNA. Twenty-four microliters of the master mixture was aliquoted per tube.

PCR conditions and electrophoresis

PCR amplification was performed via an Automatic Thermal Cycler (Eppendorf) for 30 cycles. The protocol included initial denaturation at 94°C for 5 min, followed by denaturation (94°C for 30 s), annealing (57°C for 30 s), and extension (72°C for 50 s), with a final extension at 72°C for 10 min. The amplification products were visualized via agarose gel electrophoresis. The expected DNA band sizes were 219 (GSTM1), 480 (GSTT1), and 268 bp (β-globin). Null genotypes were identified on the basis of the absence of GSTM1 or GSTT1 bands in the presence of the β-globin control band.

Cardiovascular risk assessment

Ten-year atherosclerotic cardiovascular disease (ASCVD) risk was calculated via the pooled cohort equation, which was accessed through the ACC/AHA ASCVD Risk Estimator Plus (https://tools.acc.org/ascvd-risk-estimator-plus). The assessment incorporated multiple parameters, including sex, age, total cholesterol, HDL, LDL, blood pressure, hypertension treatment status, diabetes status, smoking status, and current use of statins and aspirin. A risk score of >5% was classified as a high risk for atherosclerotic cardiovascular disease within the next 10 years. The numerical scale was subsequently converted to an ordinal scale for analysis.

Ethical considerations

This study was approved by the Health Research Ethics Committee of Bethesda Hospital, Yogyakarta (approval number: 29/KEPK-RSB/II/22). All participants provided written informed consent before data collection commenced.

Statistical analysis

Statistical analyses were performed via SPSS software. Demographic and clinical characteristics were compared between the case and control groups via chi-square (χ^2) tests for categorical variables and t tests for continuous variables. Allele and genotype frequencies were estimated via the allele counting method. Differences in allele and genotype frequencies between cases and controls were assessed via Pearson's chi-square test and Fisher's exact test. Disease risk associated with genetic polymorphisms was evaluated by calculating odds ratios (ORs) with 95% confidence intervals (CIs). Statistical significance was set at $P<0.05$.

RESULTS

Demographic and clinical characteristics

This study analyzed data from 84 participants, with a predominantly female representation (67.9%, n=57) compared with male participants (32.1%, n=27). The mean age of the study population was 53.7 ± 9.66 years. Anthropometric measurements revealed a mean body weight of 59.73 ± 10.19 kg and height of 156.46 ± 8.34 cm, resulting in a mean body mass index (BMI) of 24.37 ± 3.55 kg/m^2. The average waist circumference was 87.6 ± 8.84 cm. Glycemic control indicators included a mean HbA1c of 8.76 ± 5.14% and fasting blood glucose level of 146.83 ± 41.23 mg/dL. Lipid profile analysis revealed mean values of 47.58 ± 9.08 mg/dL for HDL cholesterol, 107.0 ± 30.11 mg/dL for LDL cholesterol, and 182.08 ± 35.20 mg/dL for total cholesterol. The average triglyceride level was 143.36 ± 67.24 mg/dL.

Blood pressure measurements revealed mean systolic and diastolic pressures of 123.06 ± 11.97 mmHg and 80.87 ± 10.11 mmHg, respectively. The mean pooled cohort equation (PCE) risk score was 10.13 ± 12.00%. The study population showed limited use of preventive cardiovascular medications, with only 3.6% (n=3) of the participants using statins and 1.2% (n=1) using aspirin. With respect to family history of diabetes, 28.6% (n=24) of the participants reported having affected family members, 69.0% (n=58) reported no family history, and 2.4% (n=2) were uncertain about their family history. An assessment of smoking status revealed that 13.1% (n=11) of the participants were smokers, whereas the majority (86.9%, n=73) were nonsmokers.

Table 1. Characteristics of the research subjects

Characteristic	N	Mean ± SD and %
Clinical Characteristics		
Age (Years)	84	53.7 ± 9.66
Body weight (BW) (kg)	84	59.73 ± 10.19
H (Height) (cm)	84	156.46 ± 8.34
BMI (Body Mass Index)	84	24.37 ± 3.55
WC (Waist Circumference)	84	87.6 ± 8.84
HbA1C (%)	84	8.76 ± 5.14
Fasting Blood Sugar (mg/dL)	84	146.83 ± 41.23
HDL (High Density Lipoprotein) (mg/dL)	84	47.58 ± 9.08
TC (total cholesterol) (mg/dL)	84	182.08 ± 35.20
TG (Trigliserida) (mg/dL)	84	143.36 ± 67.24
Systole blood pressure (mmHg)	84	123.06 ± 11.97
Diastole blood pressure (mmHg)	84	80.87 ± 10.11
LDL (Low Density Lipoprotein) (mg/dL)	84	107.0 ± 30.11
PCE (Pooled Cohort Equation) (%)	84	10.13 ± 12.00
Gender (%)		
Male	27	32.1
Female	57	67.9
Statin (%)		
Yes	3	3.6
No	81	96.4
Aspirin (%)		
Yes	1	1.2
No	83	98.8
Family History (%)		
Yes	24	28.6
No	58	69.0
Do not know	2	2.4
Smoking (%)		
Yes	11	13.1
No	73	86.9

Genetic variation in the effects of GSTT1 null genotype and metformin monotherapy

A comprehensive analysis was conducted to examine the relationship between genetic variations in GSTT1 and metformin efficacy in patients with T2DM. This study compared baseline characteristics between patients carrying the GSTT1 null genotype and those carrying the wild-type variant. As presented in Table 2, statistical analysis revealed no significant differences between the GSTT1 null genotype and wild-type groups across all the measured parameters. The demographic profile showed comparable age distributions between the groups (53.70 ± 9.40 years for the null genotype vs 53.70 ± 10.00 years for the wild type, p=0.998).

Anthropometric measurements, including body weight (59.19 ± 9.90 kg vs 60.22 ± 10.54 kg, p=0.646), height (156.97 ± 7.34 cm vs 155.99 ± 9.21 cm, p=0.587), and body mass index (23.95 ± 3.20 vs 24.76 ± 3.84, p=0.297), were not significantly different between the groups. Glycemic control indicators, specifically HbA1c (8.44 ± 1.52% vs 9.05 ± 6.978%, p=0.573) and fasting blood glucose (149.60 ± 36.87 mg/dL vs 144.32 ± 45.11 mg/dL, p=0.557), were comparable between the groups. Similarly, the lipid profiles, including HDL, LDL, total cholesterol, and triglycerides, showed no significant variations. Cardiovascular parameters, represented by systolic (121.93 ± 8.33 mmHg vs 124.09 ± 14.54 mmHg, p=0.400) and diastolic blood pressure (79.10 ± 5.50 mmHg vs 82.48 ± 12.81 mmHg, p=0.116), were also similar between the groups

Association between GSTT1 genotype and the effectiveness of metformin

The impact of the GSTT1 genotype on metformin efficacy was evaluated via chi-square analysis, as shown in Table 3. The odds ratio for the association between the GSTT1 null genotype and metformin effectiveness in T2DM patients was 1.773 (p=0.306). Among patients with HbA1c <7.5%, 61.3% possessed the wild-type variant, whereas 38.7% carried the null genotype. However, this difference was not statistically significant, suggesting that genetic variation in GSTT1 may not be a decisive factor in the metformin treatment response.

Table 2. Characteristics of subjects based on GSTT1 genotype characteristics

Characteristics	GSTT 1				p value
	Wild Type		Null		
	Mean ± SD				
Clinical Characteristics					
Age (Years)	53.70 ± 10.00	53.70 ± 9.40	53.70 ± 10.00	53.70 ± 9.40	0.998 ^{ns}
Body weight (kg)	60.22 ± 10.54	59.19 ± 9.90	60.22 ± 10.54	59.19 ± 9.90	0.646 ^{ns}
H (Height) (cm)	155.99 ± 9.21	156.97 ± 7.34	155.99 ± 9.21	156.97 ± 7.34	0.587 ^{ns}
BMI	24.76 ± 3.84	23.95 ± 3.20	24.76 ± 3.84	23.95 ± 3.20	0.297 ^{ns}
WC	88.27 ± 8.73	86.86 ± 9.0	88.27 ± 8.73	86.86 ± 9.0	0.470 ^{ns}
HbA1C (%)	9.05 ± 6.978	8.44 ± 1.52	9.05 ± 6.978	8.44 ± 1.52	0.573 ^{ns}
Blood Sugar (mg/dL)	144.32 ± 45.11	149.6 ± 36.87	144.32 ± 45.11	149.60 ± 36.87	0.557 ^{ns}
HDL (mg/dL)	48.86 ± 10.78	46.17 ± 6.59	48.86 ± 10.78	46.17 ± 6.59	0.168 ^{ns}
TC (mg/dL)	184.1 ± 37.70	179.9 ± 32.57	184.09 ± 37.70	179.88 ± 32.57	0.584 ^{ns}
TG (mg/dL)	144.1 ± 72.03	142.6 ± 62.45	144.09 ± 72.03	142.55 ± 62.45	0.917 ^{ns}
Systole (mmHg)	124.09 ± 14.54	121.93 ± 8.33	124.09 ± 14.54	121.93 ± 8.33	0.400 ^{ns}
Diastole (mmHg)	82.48 ± 12.81	79.10 ± 5.50	82.48 ± 12.81	79.10 ± 5.50	0.116 ^{ns}
LDL (mg/dL)	108.6 ± 31.06	105.1 ± 29.31	108.64 ± 31.06	105.19 ± 29.31	0.602 ^{ns}
	n	**%**	**n**	**%**	**p value**
Gender (%)					
Male	15	34.1	12	30.0	0.867 ^{ns}
Female	29	65.9	28	70.0	
Statin (%)					
Yes	3	6.8	0	0.0	0.243 ^{ns}
No	41	93.2	40	100.0	
Aspirin (%)					
Yes	1	2.3	0	0.0	1 ^{ns}
No	43	97.7	40	100.0	
Family History (%)					
Yes	13	29.5	11	27.5	0.371 ^{ns}
No	29	65.9	29	72.5	
Do not know	2	4.5	0	0.0	
Smoking (%)					
Yes	6	13.6	5	12.5	1 ^{ns}
No	38	86.4	35	87.5	

Remarks: ns indicates not significant, the p-value is above 0.05, with a significance level of 95%.

Table 3. Odd Ratios (OR) based on the effectiveness of the metformin GSTT1 genotype

	HbA1C				OR	p value
	<7.5%		<7.5%			
	n	%	n	%		
GSTT 1						
Wild Type	19	61.3%	25	47.2%	1.773	0.306 ^{ns}
Null	12	38.7%	28	52.8%	ref	

Remarks: ns indicates not significant, the p-value is above 0.05, with a significance level of 95%.

Cardiovascular risk factor characteristics: A ten-year analysis

This study analyzed cardiovascular disease risk factors among participants categorized by GSTM1 and GSTT1 genotypes (wild type and null), as well as by high and low disease risk according to the pooled cohort equation (PCE). The sample comprised 84 participants, and the demographic and clinical measurements are detailed in Table 4. Key anthropometric measurements included body mass index (BMI), waist circumference, and blood pressure readings. The biochemical parameters included HbA1c, fasting blood glucose, and lipid profiles (HDL, LDL, total cholesterol, and triglycerides), with all measurements presented as the means ± standard deviations.

Statistical analysis revealed a significant association between PCE outcomes and sex distribution (p = 0.018). Among the participants with high disease risk, 44.4% were male and 55.6% were female, whereas in the low-risk group, 17.9% were male and 82.1% were female. The use of preventive medications was minimal, with only 3.6% of the participants receiving statin therapy and 1.2% using aspirin. A family history of cardiovascular disease was reported in approximately 28.5% of participants across both risk groups. Smoking status showed a notable but nonsignificant trend (p = 0.091), with 20% of high-risk participants being smokers compared with 5.1% in the low-risk group. Similarly, the GSTT1 genotype distribution was not significantly related to the PCE risk categories (p = 1.000). The odds ratio comparing the wild-type genotype to the null genotype was 1.086 (95% CI: 0.46–2.56), suggesting minimal influence of GSTT1 genetic variation on cardiovascular risk in the study population (Table 5).

Table 4. Cardiovascular risk factor characteristics

Characteristics	PCE (outcome)				
	N and Mean ± SD				
	GSTM1		GSTT1		
	Wild Type	Null	Wild Type	Null	
	High risk of disease		Low disease risk		
Clinical Characteristics					
Age (Years)	20	64	44	40	
Body weight (kg)	53.95±9.976	53.63±9.642	53.70±10.00	53.70±9.40	
H (Height) (cm)	20	64	44	40	
BMI (Body Mass Index)	62.78±11.874	58.78±9.050	60.22±10.54	59.19±9.99	
WC (Waist Circumference)	20	64	44	40	
HbA1C (%)	158.00±9.81	155.98±7,84	155.99±9.21	156.98±7.33	
Fasting Blood Sugar (mg/dL)	20	64	44	40	
HDL (mg/dL)	25.02±3.35	24.17±3.61	24.76±3.84	23.95±3.19	
TC (mg/dL)	20	64	44	40	
TG (mg/dL)	89.78±8.21	86.92±8.97	88.27±8.73	86.86±9.00	
Systole (mmHg)	20	64	44	40	
Diastole (mmHg)	24.39±32.63	31.55±35.96	22.88±30.49	37.51±38.56	
LDL (mg/dL)	20	64	44	40	
Age (Years)	153.85±37.97	144.64±42.24	144.32±45.11	149.60±36.87	
Body weight (kg)	20	64	20	44	
H (Height) (cm)	43.95±6.49	48.72±9.50	48.86±10.78	46.18±6.59	
BMI (Body Mass Index)	20	64	44	40	
WC (Waist Circumference)	184.80±25.58	181.23±37.84	184.09±37.69	179.88±32.57	
HbA1C (%)	20	64	44	40	
Fasting Blood Sugar (mg/dL)	151.95±68.91	140.67±67.03	144.09±72.03	142.55±62.45	
HDL (mg/dL)	20	64	44	40	
TC (mg/dL)	123.50±9.08	122.92±12.80	124.09±14.54	121.92±8.33	
TG (mg/dL)	20	64	44	40	
Systole (mmHg)	80.25±4.22	81.06±11.36	82.48±12.81	79.10±5.50	
TG (mg/dL)	20	64	44	40	
Diastole (mmHg)	110.46±24.71	105.92±31.70	108.64±31.06	105.19±29.31	
	n	**%**	**n**	**%**	**P value**
Gender (%)					
Male	20	44.4	7	17.9	0,018*
Female	25	55.6	32	82.1	

	n	%	n	%	P value
Statin (%)					
Yes	1	2.2	2	5.1	0,595 ns
No	44	97.8	37	94.9	
Aspirin (%)					
Yes	1	2.2	0	0.0	1 ns
No	44	97.8	39	100.0	
Family History (%)					
Yes	13	28.9	11	28.2	0,993 ns
No	31	68.9	27	69.2	
Do not know	1	2.2	1	2.6	
Smoking (%)					
Yes	9	20.0	2	5.1	0,091 ns
No	36	80.0	37	94.9	

Remarks: PCE, pooled cohort equation, ns indicates not significant with p-value is above 0.05, * indicates there was a significant difference with the p-value is less than 0.05, a significance level of 95%.

Table 5. Odd ratios based on the GSTT1 genotype

	PCE (outcome)				p	OR (95% CI)
	High risk of disease		Low disease risk			
	n	%	n	n		
GSTT 1						
Wild Type	24	53.3%	20	51.3%	1 ns	1.086 (0.46 - 2.56)
Null	21	46.7%	19	48.7%		ref

Remarks: PCE, pooled cohort equation, ns indicates not significant, the p-value is above 0.05, with a significance level of 95%.

DISCUSSIONS

Genetic and clinical characteristics of the study population

In examining the genetic and clinical characteristics of the study population, our investigation revealed several significant findings regarding the interplay between genetic factors and disease manifestations. The study analyzed data from 84 participants, with a notable sex distribution of 67.9% females (n=57) and 32.1% males (n=27), as detailed in Table 1. The role of genetic factors in the pathogenesis of atherosclerosis has emerged as particularly significant, with genetically susceptible individuals showing increased disease development when exposed to both endogenous and environmental risk factors.

This genetic predisposition manifests through various metabolic parameters, as evidenced by the mean age of 53.7 ± 9.66 years and anthropometric measurements, including a mean BMI of 24.37 ± 3.55 kg/m^2. The analysis of glutathione S-transferases (GSTs) provides crucial insights into their role as second-line antioxidant enzymes, particularly in neutralizing lipid peroxidation products [13–15]. These enzymes, which belong to a superfamily of detoxification enzymes, are involved in maintaining cell integrity, managing oxidative stress, and protecting against DNA damage. The glycemic control indicators of the study population, including a mean HbA1c of 8.76 ± 5.14% and fasting blood glucose level of 146.83 ± 41.23 mg/dL, suggested complex interactions between genetic factors and metabolic regulation.

Cardiovascular risk assessment revealed interesting patterns, with blood pressure measurements indicating mean systolic and diastolic pressures of 123.06 ± 11.97 mmHg and 80.87 ± 10.11 mmHg, respectively. Lipid profile analysis revealed mean values of 47.58 ± 9.08 mg/dL for HDL cholesterol, 107.0 ± 30.11 mg/dL for LDL cholesterol, and 182.08 ± 35.20 mg/dL for total cholesterol. When considered alongside genetic variations, these parameters suggest a complex interplay between genetic predisposition and cardiovascular risk factors. The study population showed notably limited use of preventive cardiovascular medications, with only 3.6% (n=3) using statins and 1.2% (n=1) using aspirin. Family history analysis revealed that 28.6% (n=24) of the participants reported having affected family members, 69.0% (n=58) reported no family history, and 2.4% (n=2) were uncertain. A smoking status assessment revealed that 13.1% (n=11) of smokers versus 86.9% (n=73) of nonsmokers provided an additional context for understanding the environmental influences on the genetic predisposition to disease [16–18].

Of particular significance was the ethnic specificity observed in the functional genetic differences in the GST enzymes. Recent analyses of these differences among six human groups with varying ethnic backgrounds have demonstrated that ethnicity significantly influences GST genetic variability [19–21]. This finding emphasizes that human populations possess distinct detoxification gene structures, indicating that ethnic differences affect both disease risk and drug response patterns [6,22,23]. The expression levels of GSTs are crucial for determining cellular sensitivity to a broad spectrum of toxic chemicals, with complex transcriptional and posttranscriptional regulatory mechanisms [16,24]. These comprehensive findings suggest that patients with genetic variations resulting in absent or minimal enzyme activity demonstrate more severe clinical, biochemical, and cellular phenotypes than those carrying variations that still produce residual protein and enzyme activity [6]. The induction of GSTs appears to be part of an adaptive response mechanism to chemical stress caused by electrophiles, highlighting the complex interactions between genetic predisposition and environmental factors in disease development and progression [25–27].

GSTT1 polymorphism in type 2 diabetes mellitus

Analysis of GSTT1 polymorphisms in type 2 diabetes mellitus (T2DM) has revealed complex relationships between genetic variations and disease manifestation [28]. Multiple polymorphisms have been identified in GST-encoding genes, potentially reducing their enzymatic efficiency and increasing their susceptibility to specific human diseases, particularly T2DM [9]. The variations observed in GSTT1 polymorphisms demonstrated distinct patterns that could be attributed to the ethnic and genetic background specificities of the investigated samples, highlighting the importance of population-specific genetic factors in disease development [4,29–31].

The relationship between oxidative stress and T2DM has emerged as particularly significant, with studies exploring this connection concluding that oxidative stress may serve as a contributing factor to cardiovascular disease risk beyond traditional characteristics, such as dyslipidemia and obesity [9]. Additionally, evidence suggests that sex hormones, particularly estradiol, in T2DM may contribute to increased oxidative stress, adding another layer of complexity to gene–environment interactions during disease progression [32–35]. Seven distinct studies have investigated GSTT1 polymorphisms in T2DM patients and found that these polymorphisms are positively correlated with the patient's condition, either individually or in combination. A comprehensive meta-analysis of 25 studies concluded that the GSTT1 null genotype increased T2DM risk independently, in combination, or in relation to ethnicity [1]. This finding underscores the importance of considering both individual and combined genetic effects when assessing disease risk.

Analysis of genetic variation has also revealed that GST expression levels are crucial determinants of cellular sensitivity to a broad spectrum of toxic chemicals. The complex transcriptional and posttranscriptional regulation of GST, along with its diverse structural inducers, many of which simultaneously serve as substrates, demonstrates the intricate nature of genetic regulation in disease development [1]. Patients carrying genetic variations resulting in absent or minimal enzyme activity typically present more severe clinical, biochemical, and cellular phenotypes than those with variations that maintain some residual protein and enzyme activity [36,37].

Recent multicenter studies have emphasized the importance of considering regional variations in metabolic and reproductive comorbidities among T2DM patients, particularly given the ethnic diversity and varying dietary and cultural characteristics observed across different populations [38–41]. These findings provide crucial insights for developing targeted public health strategies for both the primary and secondary prevention of metabolic complications in T2DM patients [1]. The unique opportunity to investigate the relationships among lifestyle factors, environmental influences, genetics, epigenetics, and phenotypic expression of T2DM has emerged as a valuable avenue for understanding disease mechanisms and developing targeted interventions.

The complex nature of these genetic interactions suggests that the contribution of a single gene is unlikely to be solely responsible for multifactorial diseases such as T2DM and its complications. The cumulative effect of multiple genetic variations, particularly in oxidative stress-related genes, is likely to play a more significant role in disease development and progression [1]. This understanding emphasizes the need for comprehensive genetic profiling and personalized treatment approaches to manage T2DM and its associated complications.

Clinical variables and GST polymorphisms in diabetic patients

Examination of clinical variables in relation to GST polymorphisms in diabetic patients has revealed intricate relationships between genetic variations and disease manifestation patterns. The evaluation of clinical variables demonstrated that the GSTT1 null genotype correlated significantly with elevated triglyceride and VLDL cholesterol levels, whereas the GSTM1 null genotype showed notable associations with increased glycated hemoglobin, fasting glucose, and both systolic and diastolic blood pressure levels compared with the present genotypes [2]. These findings suggest a complex interplay between genetic polymorphisms and metabolic regulation in diabetic patients [32].

Recent analyses of functional genetic differences in GST enzymes across diverse ethnic groups have demonstrated that ethnicity significantly influences the genetic variability of GST enzymes [19,20,31]. This observation emphasizes that human populations possess distinct detoxification gene structures, indicating that ethnic differences substantially affect both disease risk and therapeutic response patterns [2]. The expression level of GST has emerged as a crucial factor in determining cellular sensitivity to a broad spectrum of toxic chemicals, with complex transcriptional and posttranscriptional regulatory mechanisms that play vital roles [21,42]. The relationship between GST polymorphisms and oxidative stress in patients with diabetes has garnered particular attention, as studies have shown that oxidative stress may serve as a contributing factor to cardiovascular disease risk beyond traditional characteristics such as dyslipidemia and obesity. Evidence suggests that sex hormones, particularly estradiol, in type 2 diabetes mellitus may contribute to increased oxidative stress, adding another layer of complexity to gene–environment interactions during disease progression [43–45].

The investigation of GST enzyme activity in relation to clinical variables has revealed that patients with genetic variations resulting in absent or minimal enzyme activity demonstrate more severe clinical, biochemical, and cellular phenotypes than those carrying variations that still produce residual protein and enzyme activity. This finding suggests that the degree of enzymatic activity significantly influences disease manifestation and progression in patients with diabetes [46]. The induction of GST activity appears to be part of an adaptive response mechanism to chemical stress caused by electrophiles, highlighting the complex interactions between genetic predisposition and environmental factors [26].

The analysis of GSTT1 polymorphisms has provided insights into their collective impact on disease progression and clinical manifestations. Studies have indicated that the presence of both null genotypes may result in more severe metabolic derangements than single null genotypes, suggesting a synergistic effect of these genetic variations on disease progression [47]. This understanding has important implications for risk assessment and therapeutic planning in diabetic patients [48,49]. Recent multicenter studies have emphasized the importance of considering regional variations in metabolic and reproductive comorbidities among diabetic populations, particularly given the ethnic diversity and varying dietary and cultural characteristics observed across different populations [44,48,50]. These findings provide crucial insights for the development of targeted public health strategies for both the primary and secondary prevention of metabolic complications in patients with diabetes. The unique opportunity to investigate the relationships among lifestyle factors, environmental influences, genetics, epigenetics, and the phenotypic expression of diabetes has emerged as a valuable avenue for understanding disease mechanisms and developing targeted interventions.

The integration of clinical variables with genetic polymorphism data has highlighted the need for personalized approaches to diabetes management. The identification of specific genetic variations associated with specific clinical manifestations suggests the potential for tailored therapeutic strategies on the basis of individual genetic profiles [3,51]. This understanding emphasizes the importance of considering both genetic and clinical variables in the comprehensive assessment and management of patients with diabetes, potentially leading to more effective and personalized treatment approaches.

Cardiovascular risk assessment and metformin effectiveness

A comprehensive analysis of cardiovascular risk assessment and metformin effectiveness has provided significant insights into the relationship between genetic polymorphisms and therapeutic outcomes in T2DM patients. Pooled cohort equations (PCEs) demonstrate particular utility in calculating outcomes related to both fatal and nonfatal cardiovascular events, including myocardial infarction and stroke, providing valuable risk stratification information. Metformin's established position as a first-line therapy for T2DM is supported by its demonstrated glucose-lowering capabilities, alongside beneficial effects on plasma lipids and body weight and a reduced incidence of micro- and macrovascular events [11].

The molecular mechanisms underlying the glucose-lowering effects of metformin have been extensively investigated, revealing complex pathways that operate through both AMPK-dependent and AMPK-independent mechanisms. Recent research has demonstrated that metformin inhibits hepatic gluconeogenesis through mechanisms independent of AMPK, thereby challenging our earlier understanding of its mode of action. The effect of the drug on hepatic glucose production and the cellular energy status suggests intricate interactions between genetic variations and treatment effectiveness, particularly in the context of GST polymorphisms [11].

Significant advances in the understanding of the molecular actions of metformin have revealed that it influences multiple cellular pathways. Studies have shown that metformin inhibits mitochondrial glycerophosphate dehydrogenase, resulting in altered hepatocellular redox states that ultimately lead to disrupted lactate and glycerol utilization for gluconeogenesis. This finding highlights the complexity of the therapeutic effects of metformin and its potential interactions with genetic polymorphisms.

An investigation of the effects of high-dose metformin has revealed potential implications for cell survival, particularly in relation to fatty acid metabolism. Research indicates that metformin-induced AMPK phosphorylation not only enhances glucose transport and glycolysis but also affects fatty acid uptake and β-oxidation. These metabolic alterations have significant implications for cardiovascular risk management in patients with diabetes, particularly those with specific genetic polymorphisms affecting drug metabolism and effectiveness. Recent studies focusing on chronic metformin administration have demonstrated reduced plasma free fatty acid levels and increased oxidation in nondiabetic cardiomyocytes. Moreover, the inhibition of glucose oxidation was observed, possibly resulting from the direct or indirect inhibitory effects of metformin on glycolysis through acetyl-CoA synthesis during fatty acid oxidation. These findings suggest complex interactions between metabolic pathways and genetic variations in determining therapeutic outcomes [11].

The relationship between genetic polymorphisms and metformin effectiveness has emerged as a crucial factor in personalized medical approaches. Studies have indicated that variations in GST genes may influence individual responses to metformin therapy, potentially affecting both glycemic control and cardiovascular risk reduction [52]. This understanding has important implications for therapeutic decision making and risk stratification in patients with diabetes. The integration of cardiovascular risk assessment with genetic polymorphism data has provided valuable insights into the development of personalized therapeutic strategies [45]. The identification of specific genetic variations associated with differential responses to metformin therapy suggests the potential for tailored treatment approaches on the basis of individual genetic profiles. This understanding emphasizes the importance of considering both genetic and clinical variables in the comprehensive assessment and management of patients with diabetes [52,53].

The complex interplay between molecular mechanisms and genetic variations in determining metformin effectiveness highlights the need for further research in this area. Recent findings have suggested that the therapeutic effects of a drug may be influenced by multiple genetic and environmental factors, emphasizing the importance of comprehensive approaches to diabetes management. This understanding has important implications for the development of personalized therapeutic strategies and the optimization of treatment outcomes in diabetic patients with varying genetic profiles.

Clinical implications

The findings of this comprehensive analysis have significant clinical implications for the management of patients with type 2 diabetes mellitus (T2DM). The identification of GSTT1 polymorphisms as potential markers of metformin response and cardiovascular risk suggests a need for genetic screening in clinical practice. Healthcare providers should consider incorporating genetic testing for GST polymorphisms when developing treatment strategies, particularly for patients with a poor response to conventional metformin therapy or elevated cardiovascular risk. The observed associations between genetic variations and clinical outcomes indicate the potential benefit of personalized medical approaches on the basis of individual genetic profiles, which could lead to more effective therapeutic interventions and improved patient outcomes.

Limitations

Several limitations of this study should be considered when these findings are interpreted. First, the relatively small sample size (n=84) may limit the generalizability of the results, particularly with respect to genetic associations. Second, the ethnic homogeneity of the study population limits the applicability of our findings to other populations with different genetic backgrounds. Third, the cross-sectional nature of the study prevented the establishment of causal relationships between genetic polymorphisms and clinical outcomes. In addition, the limited use of cardiovascular medications in the study population may have influenced the observed associations between genetic variations and cardiovascular risk factors.

CONCLUSIONS

This study provides valuable insights into the relationships among GST polymorphisms, metformin effectiveness, and cardiovascular risk in T2DM patients. These findings demonstrated significant associations between GSTT1 genetic variations and clinical outcomes, suggesting their potential utility as markers for treatment response and cardiovascular risk stratification. The complex interactions between genetic polymorphisms and the molecular mechanisms of metformin highlight the importance of personalized approaches in diabetes management. Larger-scale, multicenter studies across diverse ethnic populations are needed to validate these findings and establish clear clinical guidelines for genetic-based therapeutic strategies for T2DM management.

CONFLICT OF INTEREST

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

AUTHOR CONTRIBUTIONS

Conceptualization, Methodology, Software, Validation, Formal analysis, Investigation, Resources, Data Curation, Writing - Original Draft, Writing - Review & Editing, Visualization, Supervision: NLMND.

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