

Evaluation of glutathione S-transferase P1 genetic variants affecting type-2 diabetes susceptibility and glycemic control

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Abstract

Introduction: Type 2 diabetes mellitus (T2DM) is associated with increased production of reactive oxygen species and a reduction in antioxidant defenses leading to oxidative stress. Glutathione S-transferases (GSTs) modulate oxidative stress. The present cross-sectional study was aimed at investigating the association between the GSTP1 gene polymorphism and T2DM and to clarify their effect on the glycemic control parameters.

Material and methods: From the Egyptian population, we enrolled 112 T2DM patients and 188 healthy controls matched for age, sex and origin. Serum lipid profile, blood-glucose level, glycated hemoglobin (HbA_{1c}) and body mass index (BMI) were measured. DNA was extracted from the blood samples. Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) was used to measure GSTP1 Ile¹⁰⁵Val gene polymorphism of study participants.

Results: The frequency of the Val allele in exon 5 of the GSTP1 gene in patients with T2DM was higher than that observed in healthy controls (15.2% vs. 9.6%); the difference was considered statistically significant when compared to Ile allele carriers ($p = 0.03$). The presence of the GSTP1 heterozygous mutant allele Ile/Val was more common in subjects with T2DM than in the control group (30.4% and 19.2%, respectively; $p = 0.02$). Variation in the GSTP1 gene was associated with BMI ($p = 0.02$) and not associated with glycemic control parameters (fasting serum glucose and HbA_{1c}) or smoking-related risk of T2DM.

Conclusions: GSTP1 gene polymorphism may play a significant role in increasing the susceptibility to and risk of T2DM and obesity regardless of smoking status and had no apparent effect on HbA_{1c} in patients with diabetes mellitus.

Key words: GSTP1, type 2 diabetes mellitus, glycated hemoglobin, body mass index, polymerase chain reaction-restriction fragment length polymorphism.

Introduction

Type 2 diabetes mellitus (T2DM) represents a significant global health problem. It is estimated that six people die every minute from the disease worldwide, a figure that will soon make the disease one of the world's most prevalent causes of preventable mortality [1].

Reactive oxygen species (ROS) production induced by chronic hyperglycemia is implicated as a potential molecular mechanism behind diabetic vascular

complications. The ROS activates protein kinase C (PKC) and increases the production of advanced glycation end products (AGEs), leading to superoxide generation [2], which triggers atherosclerosis [3]. Pancreatic β -cells express low levels of anti-oxidant enzymes and become sensitive to cytotoxic stress that leads to higher risk of oxidative damage [4, 5].

Glutathione S-transferases (GSTs) belong to a group of multigene and multifunctional detoxification enzymes, which defend cells against a wide variety of toxic insults from chemicals, metabolites, and oxidative stress [6]. An important condition affecting GST expression is oxidative stress, usually observed in diabetes.

The gene expressing GST enzymes is polymorphic and therefore it is possible that individual variations in metabolic activities of each enzyme may regulate the clearance of toxic DNA intermediates and may be partially responsible for individual host susceptibility to oxidative stress damage of β -cells [7]. The GSTs comprise a class of enzymes that detoxify tobacco-related carcinogens by conjugating glutathione to facilitate their removal [8].

Improved glycemic control in people with diabetes reduces the risk of long-term complications. The Diabetes Control and Complications Trial [9] and the United Kingdom Prospective Diabetes Study [10] provided evidence for the benefits of tight and sustained glycemic control among type 1 and 2 diabetic patients. Chronic hyperglycemia confers increased risk for long-term diabetes-associated complications and repeated hemoglobin A_{1c} (HbA_{1c}) measures are a widely used marker for glycemic control in diabetes treatment and follow-up [11].

A GSTP1 variant with A to G transition in exon 5 at codon 105 leads to Ile¹⁰⁵Val amino acid substitution, which reduces the ability to conjugate reactive electrophiles with glutathione and may therefore sensitize cells to free radical-mediated damage. The Val¹⁰⁵ variant has been associated with susceptibility to smoking-related cancer [12] and cardiovascular disease [13, 14].

Studies have so far reported contradictory results regarding any association between GSTP1 gene polymorphism and T2DM [15, 16]. There has been no previous report of its effect on the glycemic control parameters. Thus, we conducted a case-control study in the Egyptian population with age- and sex-matched T2DM to evaluate whether the GSTP1 variants modulate the risk of T2DM patients and to clarify their effect on the glycemic control parameters.

Material and methods

Subjects

A total of 112 T2DM patients and a control group of 188 healthy subjects were examined in this study.

Diagnosis of T2DM was based on the American Diabetes Association definition of diabetes [17]. Healthy subjects did not meet the criteria for the diagnosis of diabetes mellitus.

Laboratory measurements

Venous blood samples were drawn after an overnight fast. HbA_{1c} was measured with an ADVIA 1800 chemistry analyzer (Siemens Healthcare Diagnostics, USA). Fasting serum glucose, serum total cholesterol, HDL cholesterol and triglycerides (TG) were measured by automated enzymatic methods on an Hitachi-912 analyzer; LDL cholesterol was calculated according to the Friedewald formula [18]. Hypertension was defined as systolic blood pressure (BP) \geq 140 mm Hg, diastolic BP \geq 90 mm Hg and/or a history of hypertension [19]. Current smoker was defined as a subject who continued to smoke cigarettes regularly.

Study subjects

All the procedures were carried out according to the principles of the Declaration of Helsinki. The study was approved by the Suez Canal University Research Ethics Committee and all the patients and controls provided written informed consent. Table I summarizes the clinical features of patients and controls.

Determination of genotype at the GSTP1 locus

Genomic DNA was obtained from peripheral blood samples collected in EDTA tubes. The DNA was extracted using DNA purification kit cat #1120 (Promega, USA). The assay for the polymorphisms in GSTP1-105 was performed as described previously [20]. Each PCR reaction mixture (40 μ l) contained 200 ng each of primers P105F (5'-ACC CCA GGG CTC TAT GGG AA-3') and P105R (5'-TGA GGG CAC AAG AAG CCC CT-3'), 100 ng of genomic DNA, 1.5 mM MgCl₂, 100 mM of each dNTP and 1 U Taq polymerase (Promega, USA). Initial denaturation was carried out at 95°C for 5 min. The reaction involved 30 cycles of incubation at 94°C (30 s), 55°C (30 s), and 72°C (30 s). A final polymerization step of 72°C for 5 min was carried out to complete the elongation processes. After the confirmation of an amplified fragment of the expected size (176 bp) on an agarose gel, the PCR products were digested with 5 U of restriction enzyme Alw261 (Fermentas, UK) in a total volume of 25 μ l. DNA fragments were submitted to electrophoresis through a 3.5% agarose gel and stained with ethidium bromide (10 mg/ml).

Statistical analysis

All data was entered, independently verified and analyzed using SPSS Statistics 17.0. Chi-squared statistics were performed to assess differences

Table I. General characteristics of the study population

Variable	Control (n = 188)	T2DM (n = 112)	Value of p
Age [years]	48.54 ±8.07	48.67 ±7.3	0.89
Sex (M/F)	101/87	60/52	0.98
FBGL level [mg/dl]	96.16 ±8.8	208.5 ±95.1	0.0001*
HbA _{1c}	5 ±0.74	9.67 ±1.89	0.0001*
BMI [kg/m ²]	28.23 ±3.94	29.42 ±4.08	0.013*
Total cholesterol [mg/dl]	210.33 ±85.05	217.69 ±46.13	0.39
TG [mg/dl]	151.52 ±77.09	168.56 ±89.45	0.82
HDL-C [mg/dl]	46.91 ±11.19	45.16 ±10.78	0.18
VLDL-C [mg/dl]	30.3 ±15.42	33.7 ±17.89	0.08
LDL-C [mg/dl]	133.11 ±85.62	138.82 ±47.45	0.52
Current smoking (+/-)	35/153	28/84	0.89
Systolic BP [mm Hg]	120.29 ±15.52	142.99 ±25.34	0.0001*
Diastolic BP [mm Hg]	75.13 ±8.03	83.21 ±12.23	0.0001*

FBGL – fasting blood glucose level [mg/dl]. Comparisons were performed by Student *t*-test and χ^2 test; data are mean ± SD. *Significant differences between groups ($p < 0.05$)

between case and control populations in relation to non-continuous variables. Student's *t*-test was used to test differences based on continuous variables.

Results

A total of 300 subjects were enrolled in this study (112 T2DM patients and 188 gender- and age-matched controls). The basic demographic data, including body mass index (BMI), age, gender and clinical characteristics of the study populations are summarized in Table I. No significant difference was found between patients and controls in age (45.67 ±7.3 vs. 48.54 ±8.07) or gender (male: female ratio = 60 : 52 vs. 101 : 87) respectively. In diabetics the established risk factors (hypertension, BMI, HbA_{1c} and fasting blood glucose level) for diabetes were higher than in control subjects. On the other hand, no significant difference in the lipid profiles was observed among the studied groups.

GSTP1 alleles in the two groups

There was no deviation in the distribution of GSTP1 exon 5 polymorphism genotypes from Hardy-Weinberg equilibrium in the groups studied. The results of exon 5 variants of GSTP1 evaluated by comparing the T2DM group with controls are listed in Table II.

The G allele (Val) was more prevalent among patients (15.2%) than among control subjects (9.6%) ($p = 0.03$). We found that 30.4% of the diabetic patients and 19.2% of the control subjects were heterozygous for the G allele (Ile/Val) ($p = 0.02$) and G allele homozygosity (Val/Val) could not be detect-

ed in any of the control subjects or the patients. Compared with the Ile/Ile genotype, subjects with the Ile/Val genotype had an elevated risk of type 2 diabetes mellitus (OR = 1.84, 95% CI = 1.07-3.17). The risk was increased with the G allele frequency ($p = 0.03$) (Figure 1).

Clinical and functional characteristics in relation to GSTP1 genotypes

The correlation between different genotypes of exon 5 of the GSTP1 gene with clinical and functional parameters is presented in Table III. We found no statistically significant interactions between GSTP1 genotypes and the age of onset of diabetes ($p = 0.27$). There was no significant influence of these genotypes on lipid profile, smoking or blood pressure. On the other hand, there was a significant influence of these genotypes on BMI ($p = 0.02$).

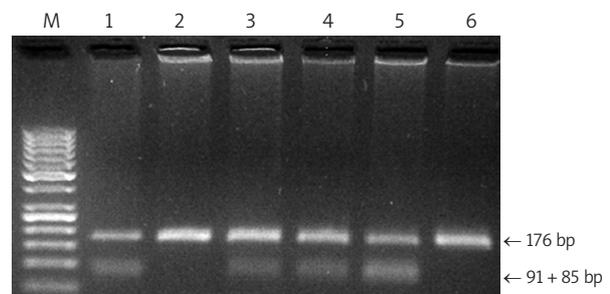


Figure 1. Genotyping of GSTP1 Ile¹⁰⁵Val by PCR-RFLP on 3.5% agarose gel, which distinguishes between the GSTP1 genotypes: A/A (Ile/Ile) – lines 2 and 6 (176 bp); A/G (Ile/Val) – lines 1 and 3-5 (176, 91 and 85 bp)

Table II. Frequencies and genotype distribution of GSTP1 gene A/G polymorphism in control and type II diabetic patients

GSTP1 gene	Control, n (%), n = 188	T2DM, n (%), n = 112	χ^2	Value of p	OR	95% CI
A allele (Ile)	340 (90.4)	190 (84.8)				
G allele (Val)	36 (9.6)	34 (15.2)	4.27	0.03*	1.69	1.024-2.78
AA (Ile/Ile)	152 (80.8)	78 (69.6)				
AG (Ile/Val)	36 (19.2)	34 (30.4)	4.92	0.02*	1.84	1.07-3.17
GG (Val/Val)	0 (0)	0 (0)				

χ^2 – Chi square test, CI – confidence interval, OR – odds ratio. *G vs. A, **AG vs. AA. Value of p < 0.05 is statistically significant

Table III. The relationship between GSTP1 genotypes with different clinical parameters in diabetic patients

Variables	Carriers of AA (n = 78)	Carriers of AG (n = 34)	Value of p
Onset age [years]	5.17 ±2.05	5.62 ±1.84	0.27
Sex (M/F)	33/45	12/22	0.49
FBGL	207.38 ±23.93	211.08 ±99.06	0.85
HbA _{1c} [%]	9.7 ±1.96	9.59 ±1.77	0.78
BMI	28.83 ±4.21	30.76 ±3.47	0.02*
Total cholesterol [mg/dl]	214.03 ±45.77	226.12 ±46.5	0.2
TG [mg/dl]	174.13 ±92.02	155 ±79 ±83.18	0.32
HDL-C [mg/dl]	45.56 ±11.65	44.24 ±8.52	0.55
VLDL-C [mg/dl]	34.83 ±18.4	31.16 ±16.64	0.32
LDL-C [mg/dl]	133.64 ±47.05	150.72 ±46.88	0.08
Current smoking (+/-)	20/58	8/26	0.81
Systolic BP	144.74 ±23.93	138.97 ±28.28	0.27
Diastolic BP	82.82 ±11.83	84.12 ±13.23	0.6

Comparisons were performed by Student-t test and χ^2 test; data are mean ± SD. *Significant differences between groups (p < 0.05)

The association of polymorphism in GSTP1 with glycemetic control parameters of diabetes mellitus

Diabetic control is expressed by glycosylated hemoglobin (HbA_{1c}) and fasting serum glucose level. No effect of polymorphism in the GSTP1 gene on glycemetic control parameters was found. Glycosylated hemoglobin was 9.59 ±1.77 in Ile/Val genotype of the GSTP1 gene and 9.7 ±1.96 in Ile/Ile genotype of the GSTP1 gene, with p = 0.78.

Discussion

Oxidative stress has been considered to be a common pathogenic factor in diabetes and its complications [21]. The family of GST genes plays an important role in protecting cells from oxidative stress. GSTP1 catalyses the detoxification of products arising from DNA oxidation [22]. A defect in detoxifying reactive oxygen species, which is genetically determined, may influence the development and severity of diabetes mellitus [16].

There are many studies dealing with GSTP1 polymorphism in various diseases, but only a few studies have addressed the role of GSTP1 gene polymorphism in diabetes. Therefore, the current study was designed to investigate the role of GSTP1 (Ile¹⁰⁵Val) gene polymorphism in T2DM patients and healthy controls and whether this variant modulates the glycemetic control in Egyptian patients with T2DM.

Our results demonstrate that the frequency of the G allele was higher in the diabetics compared to controls (OR = 1.69, 95% CI = 1.024-2.78, p = 0.03). Additionally, significant differences in the frequencies of the Ile/Val genotype between patients and the control group were observed (30.4% vs. 19.2% respectively). We therefore suggest that the G allele (Val) of GSTP1 Ile¹⁰⁵Val plays an important role in predisposition to T2DM.

There have been controversial results regarding the association between GSTP1 Ile¹⁰⁵Val gene polymorphism and diabetes development. We are in agreement with Ramprasath *et al.* [15] and Bid *et al.* [23], who demonstrated that the GSTP1 G allele

(Val) and its variant genotype (Ile/Val) play a vital role in the development of diabetes mellitus. In contrast, Yalin *et al.* [16] and Oniki *et al.* [24] suggested that GSTP1 Ile¹⁰⁵Val polymorphism may not play a significant role in the etiopathogenesis of DM in the Turkish population and Japanese population respectively. These inconsistent data could be explained by ethnic differences in the selected study groups [25].

To investigate the association between GSTP1 Ile¹⁰⁵Val gene polymorphism and glycemic control in relation to T2DM we evaluated HbA_{1c} and serum glucose levels in different genotypes. No association between the gene polymorphism and glycemic control parameters in T2DM patients was detected. To our knowledge, this study is the first attempt to evaluate the effect of the GSTP1 genotypes with regard to glycemic control parameters in T2DM.

The epidemic of T2DM observed in recent years is a clear indication of the importance of environmental factors in diabetes onset, in particular obesity and physical inactivity [23]. Obesity, mainly when fat is distributed predominantly at the abdominal level, is the main risk factor for T2DM. Our study is probably the first study to show that GSTP1 heterozygosity (Ile/Val) is significantly associated with BMI.

Dyslipidemia observed in T2DM is one of the major factors contributing to vascular risk [26]. In the current study, we further investigated the effect of the genotypes on the lipid profile. There was no association between the genotypes and lipid profile in diabetic patients. This data are consistent with the previous reports which demonstrated that there was no association between GSTP1 genotypes and blood lipids in T2DM patients [15, 23].

GSTP1 detoxifies cigarette smoke-derived toxins and endogenously derived reactive oxygen species. Therefore, it is conceivable that genetic polymorphism in GSTP1 may have an effect on smoking-related risk in T2DM. Concerning the relation to smoking status, previous report showed that active smoking is associated with an increased risk of T2DM [27]. We found that GSTP1 polymorphism was not significantly linked with smoking in T2DM patients. These data are in accordance with Oniki *et al.* [24].

In conclusion, this is the first study to determine the association of type 2 diabetes with GSTP1 Ile¹⁰⁵Val gene polymorphism in the Egyptian population and its effect on glycemic control parameters. These results show that GSTP1 Ile/Val genotype may play a significant role in the etiopathogenesis of T2DM, and the GSTP1 gene may be a useful marker in the prediction of T2DM susceptibility of the Egyptian population regardless of smoking status. Our findings suggest that the GSTP1 gene polymorphism had no apparent effect on glycemic control in type 2 diabetes patients; meanwhile, there was a significant

correlation with BMI in diabetics. Although some of our data were statistically significant, we acknowledge that the findings presented here are preliminary because of the small number of subjects and that the study requires confirmation in a separate, larger cohort. Additionally, a wide epidemiological study is needed to test the possible association between genotypic and phenotypic effects of other GST gene polymorphisms in diabetic patients.

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